CTD 第 2 部

2.6　非臨床試験の概要文及び概要表

2.6.4　薬物動態試験の概要文

MSD 株式会社

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略号及び用語の定義

略号	省略していない名称（英語）	省略していない名称（日本語）
ADME	Absorption, distribution, metabolism, and excretion	吸収、分布、代謝及び排泄
AMS	Accelerator mass spectrometry	加速器質量分析法
AUC	Area under the concentration-time curve	血漿中濃度-時間曲線下面積
BCRP	Breast cancer resistance protein	乳癌耐性蛋白
BDC	Bile duct cannulation	胆管カニュレーション
BSP	Sulfobromophthalein	スルホブロモフタレイン
CL_P	Plasma clearance	血漿クリアランス
C_{max}	Maximum concentration	最高血漿中濃度
CYP	Cytochrome P450	チトクロム P450
DDI	Drug-Drug Interaction	薬物相互作用
DPP-4	Dipeptidyl Peptidase 4	ジペプチジルペプチダーゼ4
F	Absolute oral bioavailability	絶対的経口バイオアベイラビリティ
GLP	Good Laboratory Practice	医薬品の安全性に関する非臨床試験の実施の基準
HPLC	High performance liquid chromatography	高速液体クロマトグラフィー
IC_{50}	50% Inhibitory concentration	50%阻害濃度
IV	intravenous	静脈内
KO	Knock-out	ノックアウト
LC-MS/MS	Liquid chromatography-tandem mass spectrometry	液体クロマトグラフィータンデム質量分析
LLC-PK	Lilly laboratory cells-porcine kidney	ブタ腎臓近位尿細管由来上皮細胞株
MATE	Multidrug and toxin extrusion	－
MDCK	Madin-darby canine kidney	イヌ腎臓尿細管上皮細胞由来細胞株
MDR	Multiple dug resistance protein	多剤耐性蛋白
MEC	Molar extinction coefficient	モル吸光係数
mRNA	Messenger ribonucleic acid	伝達リボ核酸
MRT	Mean residence time	平均滞留時間
NADPH	β-Nicotinamide adenine dinucleotide phosphate, reduced form	（還元型）β-ニコチンアミドアデニンジヌクレオチドリン酸
N.A.	Not analyzed	解析不能
ND	Not determined	検出不能
hOAT	Human organic anion transporter	ヒト有機アニオントランスポーター
OATP	Organic anion transporting polypeptide	有機アニオン輸送ポリペプチド
hOCT	Human organic cation transporter	ヒト有機カチオントランスポーター
P_{app}	Apparent permeability coefficients	見かけの透過係数
P-gp	P-glycoprotein	P-糖蛋白
P.O.	Per os	経口
QWBA	Quantitative whole body autoradiography	定量的全身オートラジオグラフィー
SD	Standard deviation	標準偏差
SULT	Sulfotransferase	硫酸転移酵素
$t_{1/2}$	Half-life	見かけの終末相半減期
TFA	Trifluoroacetic acid	トリフルオロ酢酸
T_{max}	Time to the maximum concentration	最高濃度到達時間
UGT	Uridine diphosphate-glucuronosyltransferase	UDP-グルクロン酸転移酵素
Vd_{ss}	Volume of distribution at steady state	定常状態分布容積

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2.6.4.1　まとめ

オマリグリプチン（別名 MK-3102又は L-001952824。以下 MK-3102）は、2型糖尿病の治療を目的として開発された長時間作用型ジペプチジルペプチダーゼ-4（DPP-4）選択的阻害薬である。本項では、非臨床毒性評価に選択した動物2種、Sprague Dawley ラット及びビーグル犬におけるMK-3102の吸収、分布、代謝及び排泄（ADME）を示す。実施した非臨床 ADME 試験を薬物動態試験一覧表にまとめた[2.6.5.1 項]。また、CD1マウス及び Dutch Belted ウサギの肝ミクロソームを用いて、MK-3102の in vitro 代謝を検討した。がん原性試験にはマウスを、催奇形性試験にはウサギをそれぞれ使用した。ヒトにおける蛋白結合率、代謝及び排泄について検討し、非臨床動物モデルとヒトの種差を比較した。

非臨床 ADME 試験において、ラット及びイヌでは MK-3102の血漿クリアランスは低く、半減期が長いことが示された。また、ラット、イヌのいずれにおいても MK-3102は速やかに吸収され、絶対的経口バイオアベイラビリティが高いことが示された。経口投与による有色 Long-Evans ラットの組織分布試験では、[^{14}C]-MK-3102由来の放射能は速やかに吸収され、脳や精巣をはじめとする組織に広範に分布した。一部組織では放射能濃度が測定可能であり、経時的に減衰し、最終試料採取時点ではごく微量となった（投与672時間後で2%未満）。[^{14}C]-MK-3102由来の放射能は、メラニンを含有する有色組織に親和性を示した。MK-3102は、ラット及びウサギの胎盤を通過し、授乳期ラットの乳汁に移行した。非臨床試験で用いた動物種とヒトの血漿蛋白結合率には種差が認められ、平均非結合型分率は、ラットでは15%、カニクイザルでは87%と幅広かった。MK-3102は、臨床相当濃度（1～1000 nM）では、ヒト血漿蛋白に対して濃度依存的な結合を示し、これはDPP-4との結合の飽和に起因するものであった。MK-3102は、ラットやイヌのほかにヒトでも主に腎排泄により消失し、代謝の寄与は小さかった。ヒト尿では、ごく微量の酸化代謝物が数種類検出された。これらの代謝物はラット尿でも検出された。ヒト血漿では未変化体が血中放射能のほぼすべてを占め、主要な血中代謝物は検出されなかった。以上をまとめると、ヒトで確認されたすべての代謝物が非臨床試験で用いた動物種においても検出された。

In vitro データから、MK-3102が薬物相互作用を引き起こす可能性は低いことが示されており、臨床試験でこの結果が確認された。臨床相当濃度（1～1000 nM）では、MK-3102はチトクロム P450（CYP1A2、2B6、2C8、2C9、2C19、2D6及び3A4）、第 II 相酵素［UDP-グルクロン酸転移酵素（UGT）1A1及び硫酸転移酵素（SULT）1E1］のほか、ヒト P-糖蛋白（P-gp）、ヒト乳癌耐性蛋白（BCRP）、ヒト有機アニオン輸送ポリペプチド1B1及び1B3（OATP1B1及び1B3）、ヒト有機アニオントランスポーター1及び3（hOAT1及び hOAT3）、ヒト有機カチオントランスポーター1及び2（hOCT1及び hOCT2）並びにヒト multidrug and toxin extrusion 1（MATE1）などのヒト主要薬物トランスポーターを阻害せず、IC_{50}値は50 μM 超であった。さらに、MK-3102は CYP3A4、CYP1A2及び CYP2B6を誘導しなかった。MK-3102の腎排泄には、受動的透過による再吸収が関与していると考えられる。また、MK-3102は、近位尿細管に発現しているトランスポーター（P-gp、hOAT1、hOAT3及び hOCT2）又は肝取込みトランスポーター（hOCT1）の基質ではなかった。以上のことから、MK-3102が薬物相互作用を引き起こす、又は受けるリスクは概して低いと考えられる。

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2.6.4.2　分析法

ラット及びイヌを用いた PK 試験では、定量下限1 ng/mL（0.002 μM）の探索的液体クロマトグラフィータンデム質量分析（LC-MS/MS）法を用いて、血漿中 MK-3102濃度を測定した。毒性試験の血漿中又は乳汁中の MK-3102濃度測定にはいずれもバリデートされた LC-MS/MS 法を用いた［定量下限は、サル試験では20.0 ng/mL（0.0502 μM）、そのほかの試験では10.0 ng/mL（0.0251 μM）］。バリデーション報告書[資料4.2.2.1: PK016]に記載したとおり、「医薬品の安全性に関する非臨床試験の実施の基準」（GLP）ガイドラインに準拠してバリデーションを実施しており、最大4000 ng/mL まで良好な直線性が確認された。試験に適用したパラメータをはじめとする生体試料中濃度分析法の詳細を[表2.6.4: 1]に示した。

ラット及びイヌを用いた体内動態試験では、直接液体シンチレーション計数法を用いて胆汁、尿及び血漿中の放射能濃度を測定した。糞中放射能濃度は糞ホモジネートを可溶化した後、液体シンチレーション計数法により測定した。ラジオクロマトグラムによる代謝物プロファイリングには、放射能測定用検出器の付いた逆相高速液体クロマトグラフィー（HPLC）を使用した。ヒト ADME 試験では、加速器質量分析法（AMS）により、血漿、尿及び糞試料中の放射能濃度を測定した。ヒトの排泄物及び血漿試料の代謝物プロファイリングには HPLC で分画した後、AMS により各画分の放射能を測定し、ラジオクロマトグラムを作成した。代謝物の構造は質量分析によって明らかにし、代謝物の標準物質がある場合には標準物質との比較を実施した。

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表 2.6.4: 1　　バリデーション済みの生物学的分析法と試験詳細

試験番号	動物種／系統	GLP	分析対象化合物	LC-MS/MS 法詳細
TT#[redacted]1080 TT#[redacted]6015 TT#[redacted]1007	イヌ／ビーグル イヌ／ビーグル イヌ／ビーグル	適用 適用 適用	MK-3102 MK-3102 MK-3102	マトリックス：イヌ血漿 試料前処理：蛋白沈殿 試験範囲：10.0～4000 ng/mL バリデーション方法：SBP 369
TT#[redacted]1081 TT#[redacted]6014 TT#[redacted]1006 TT#[redacted]7000 TT#[redacted]1166 TT#[redacted]7130 TT#[redacted]9003	ラット／SD ラット／SD ラット／SD ラット／SD ラット／SD ラット／SD ラット／SD	適用 適用 適用 適用 適用 適用 適用	MK-3102 MK-3102 MK-3102 MK-3102 MK-3102 MK-3102 MK-3102	マトリックス：ラット血漿 試料前処理：蛋白沈殿 試験範囲：10.0～4000 ng/mL バリデーション方法：SBP 369
TT#[redacted]7130	ラット／SD	適用	MK-3102	マトリックス：ラット乳汁 試料前処理：蛋白沈殿 試験範囲：10.0～4000 ng/mL バリデーション方法：SBP 369
TT#[redacted]6006 TT#[redacted]6010	サル／カニクイザル サル／カニクイザル	非適用 適用	MK-3102 MK-3102	マトリックス：サル血漿 試料前処理：蛋白沈殿 試験範囲：20.0～4000 ng/mL バリデーション方法：SBP 369
TT#[redacted]6035 TT#[redacted]6037 TT#[redacted]1061 TT#[redacted]1002	マウス／rasH2 野生型 マウス／CD1 マウス／CD1 マウス／CD1	適用 適用 適用 適用	MK-3102 MK-3102 MK-3102 MK-3102	マトリックス：マウス血漿 試料前処理：蛋白沈殿 試験範囲：10.0～4000 ng/mL バリデーション方法：SBP 369
TT#[redacted]7287 TT#[redacted]7010 TT#[redacted]7140	ウサギ／Dutch Belted ウサギ／Dutch Belted ウサギ／Dutch Belted	非適用 適用 適用	MK-3102 MK-3102 MK-3102	マトリックス：ウサギ血漿 試料前処理：蛋白沈殿 試験範囲：10.0～4000 ng/mL バリデーション方法：SBP 369

[資料 4.2.2.1: PK016]

2.6.4.3　吸収

2.6.4.3.1　単回投与

2.6.4.3.1.1　ラット

雄 Sprague Dawley ラットに MK-3102（1 mg/kg）を静脈内投与したところ、血漿クリアランス（CL_p）及び分布容積（Vd_{ss}）の平均値、並びに平均滞留時間（MRT）は、それぞれ1.1 mL/min/kg、0.77 L/kg 及び11時間であった[表2.6.4: 2]。MK-3102は、ラットにおいてクリアランスが低いという特徴を示した。

経口投与後（2 mg/kg）、MK-3102は速やかに吸収され、投与0.5～2時間後に最高血漿中濃度（C_{max}）に到達した[表2.6.4: 2]。絶対的経口バイオアベイラビリティ（F）は約100%と推定され、MK-3102がほぼ完全に吸収されていることを示している。このことは、MK-3102が溶解性及び膜透過性が高い化合物であることとも一致している[2.7.1.1.1 項][2.6.4.4.5 項]。

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表 2.6.4: 2　　ラットに静脈内及び経口投与後の MK-3102 の薬物動態パラメータ [a]

用量 (mg/kg)	投与経路	$AUC_{0-\infty}$ (μM·hr)	CL_p (mL/min/kg)	Vd_{ss} (L/kg)	MRT (hr)	C_{max} (μM)	T_{max} (hr)	F[b] (%)
1	静脈内	38.3	1.1	0.77	11	—	—	—
2	経口	95.5 (18.5)	—	—	—	8.96 (1.11)	0.5-2	約100

[a] 静脈内投与試験及び経口投与試験では、MK-3102遊離体をエタノール：水（5:95）で溶解して液剤に調製した。T_{max}（表に記載の範囲）を除き、数値は動物3例の平均値及び SD 又は2例の平均値である。なお、静脈内投与及び経口投与した際、MK-3102の血漿中濃度推移の最終消失相に2番目のピークがみられ、消失相を適切に捉えることができなかったため、$t_{1/2}$を算出しなかった。
[b] 静脈内投与後の $AUC_{0-\infty}$の平均値及び経口投与後の個体別の $AUC_{0-\infty}$値を用いて F を算出した。

[2.6.5.3 項]

2.6.4.3.1.2　イヌ

MK-3102を1 mg/kg で静脈内投与したイヌでは、CL_p、Vd_{ss} 及び $t_{1/2}$の平均値がそれぞれ、0.9 mL/min/kg、1.3 L/kg 及び22時間であった[表2.6.4: 3]。MK-3102は、イヌにおいてクリアランスが低いという特徴を示した。

2 mg/kg を経口投与後、MK-3102は速やかに吸収され、T_{max} は投与0.5～2時間後であった。絶対的経口バイオアベイラビリティは約100%であり、MK-3102がほぼ完全に吸収されることを示している[表2.6.4: 3]。

表 2.6.4: 3　　イヌに静脈内及び経口投与後の MK-3102 の薬物動態パラメータ [a]

用量 (mg/kg)	投与経路	$AUC_{0-\infty}$ (μM·hr)	CL_p (mL/min/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (hr)	C_{max} (μM)	T_{max} (hr)	F[b] (%)
1	静脈内	47.1	0.9	1.3	22	—	—	—
2	経口	108	—	—	—[c]	5.94	0.5-2	約100

[a] 静脈内投与試験及び経口投与試験では、生理食塩水に MK-3102TFA 塩を溶解して液剤に調製した。T_{max}（表に記載の範囲）を除き、数値は2例の平均値である。
[b] 静脈内投与後の $AUC_{0-\infty}$の平均値及び経口投与後の個々の $AUC_{0-\infty}$値を用いて F を算出した。
[c] MK-3102の吸収は速いことを考慮すると、静脈内投与時の半減期と一貫していると考えられるので、経口投与時の $t_{1/2}$は算出しなかった。

[2.6.5.3 項]

2.6.4.3.2　反復投与

2.6.4.3.2.1　ラット

ラット反復投与毒性試験において、初回投与時の TK パラメータを算出していないため、別途実施した単回投与試験（PK001試験）で MK-3102 2 mg/kg 単回経口投与した際の $AUC_{0-24\ hr}$及び C_{max}と14日間及び13週間反復投与した際の $AUC_{0-24\ hr}$及び C_{max}を比較した[2.6.4.3.1.1 項]。[表2.6.4: 2] に示すラット単回投与時の $AUC_{0-\infty}$は、$AUC_{0-48\ hr}$を記載しているため、比較のため $AUC_{0-24\ hr}$（79.1 μM·hr）を再計算した。

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14日間経口投与毒性試験での蓄積係数（第14日／第1日）は、$AUC_{0\text{-}24\ hr}$で1.10～1.39、C_{max}で0.95～1.16であった[表2.6.4: 4]。6ヵ月間経口投与毒性試験（13週目の TK パラメータを算出）での蓄積係数（第13週／第1日）は、$AUC_{0\text{-}24\ hr}$で1.38～1.71、C_{max}で1.10～1.15であった[表2.6.4: 5]。

単回投与試験では2 mg/kg 以外の投与量で実施していないため、反復投与毒性試験での2 mg/kg 以外の投与量での蓄積係数は算出しなかった。しかし、10 mg/kg/日で反復投与した際の曝露は、14日間経口投与毒性試験では $AUC_{0\text{-}24\ hr}$ 433 μM·hr、C_{max} 43 μM、6ヵ月間経口投与毒性試験では $AUC_{0\text{-}24\ hr}$ 534 μM·hr、C_{max} 45.4 μM であり、7日間経口投与忍容性試験での $AUC_{0\text{-}24\ hr}$ 519 μM·hr、C_{max} 48 μM [2.6.6.3.3 項]と類似していた。異なる投与期間で曝露が類似していたことから、投与7日目以降、13週目までさらなる蓄積はないことが示された。

表 2.6.4: 4　　ラットの 2 週間毒性試験における MK-3102 の TK パラメータ：試験 2 週

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (μM·hr)		C_{max} (μM)		T_{max} (hr)
		Day 14	Ratio (Day 14/Day 1†)	Day 14	Ratio (Day 14/Day 1†)	Day 14
2	Female	110 ± 4.29	1.39	10.4 ± 0.377	1.16	0.5 ± NC
	Male	87.2 ± 6.52	1.10	8.49 ± 0.465	0.95	1.0 ± NC
	All	98.4 ± 4.86	1.24	9.20 ± 0.464	1.03	1.0 ± NC

Data on Day 14 are represented as the mean ± standard error (n=10 for each sex, n=20 for all)
NC: Not Calculated
†1日目の曝露は単回投与試験 [2.6.4.3.1.1 項]で得られた $AUC_{0\text{-}24\ hr}$ 79.1μM·hr 及び C_{max} 8.96 μM を用いた。
[2.6.6.3.4 項] [2.6.7.7A 項] Fourteen-Day Oral Toxicity Study in Rats with Functional Observational Battery (FOB) (TT#[redacted]-1081)

表 2.6.4: 5　　ラットの 6 ヵ月毒性試験における MK-3102 の TK パラメータ：試験 13 週

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (μM·hr)		C_{max} (μM)		T_{max} (hr)
		Week 13	Ratio (Week 13/Day 1†)	Week 13	Ratio (Week 13/Day 1†)	Week 13
2	Female	135 ± 3.90	1.71	10.3 ± 0.290	1.15	2.0 ± NC
	Male	109 ± 6.79	1.38	9.86 ± 0.745	1.10	2.0 ± NC
	All	122 ± 5.65	1.54	10.1 ± 0.378	1.13	2.0 ± NC

Data are represented as the mean ± standard error (n=15 for each sex, n=30 for all)
NC: Not Calculated
†1日目の曝露は単回投与試験 [2.6.4.3.1.1 項]で得られた $AUC_{0\text{-}24\ hr}$ 79.1μM·hr 及び C_{max} 8.96 μM を用いた。
[2.6.6.3.6 項] [2.6.7.7C 項] Six-Month Oral Toxicity Study in Rats (TT#[redacted]-1006)

2.6.4.3.2.2　イヌ

14日間経口投与毒性試験において、MK-3102を5 mg/kg/日の用量でビーグルに14日間経口投与した際の蓄積係数（第14日／第1日）は、$AUC_{0\text{-}24\ hr}$で1.23～1.81、C_{max}で1.54～1.67であった[表2.6.4: 6]。また、9ヵ月経口投与毒性試験（投与13週目に TK パラメータを算出）において、MK-3102を

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2、10及び75 mg/kg/日で経口投与した際の蓄積係数（第13週／第1日）は、$AUC_{0\text{-}24\ hr}$で1.38～2.44、C_{max}で1.12～1.83であった[表2.6.4: 7]。

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表 2.6.4: 6　　イヌの 2 週間毒性試験における MK-3102 の TK パラメータ：試験 2 週

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (μM·hr)			C_{max} (μM)			T_{max} (hr)	
		Day 1	Day 14	Ratio (Day 14/Day 1)	Day 1	Day 14	Ratio (Day 14/Day 1)	Day 1	Day 14
5	Female	137 ± ID	169 ± ID	1.23 ± ID	10.1 ± ID	16.8 ± ID	1.67 ± ID	1.5 ± ID	1.0 ± ID
	Male	120 ± ID	239 ± ID	1.81 ± ID	9.84 ± ID	15.4 ± ID	1.54 ± ID	1.5 ± ID	4.5 ± ID
	All	128 ± 16.3	204 ± 60.6	1.52 ± 0.30	9.97 ± 0.425	16.1 ± 2.20	1.60 ± 0.17	1.5 ± 0.29	2.8 ± 1.8

Data are represented as the mean ± standard error (n=2 for each sex, n=4 for all)
ID: Insufficient Data
[2.6.6.3.7 項] [2.6.7.7D 項] Fourteen-Day Oral Toxicity Study in Dogs (TT#[redacted]-1080)

表 2.6.4: 7　　イヌの 9 ヵ月毒性試験における MK-3102 の TK パラメータ：試験 13 週

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (μM·hr)			C_{max} (μM)			T_{max} (hr)	
		Day 1	Week 13	Ratio (Week 13/Day 1)	Day 1	Week 13	Ratio (Week 13/Day 1)	Day 1	Week 13
2	Female	52.8 ± 7.92	69.9 ± 9.76	1.46 ± 0.10	4.29 ± 0.558	5.55 ± 0.983	1.31 ± 0.04	1.0 ± 0.0	2.7 ± 0.67
	Male	54.1 ± 5.28	108 ± 14.3	2.00 ± 0.22	5.37 ± 0.385	7.97 ± 1.22	1.46 ± 0.16	1.0 ± 0.0	1.3 ± 0.43
	All	53.5 ± 4.41	91.5 ± 11.4	1.77 ± 0.16	4.83 ± 0.374	6.93 ± 0.896	1.40 ± 0.09	1.0 ± 0.0	1.9 ± 0.45
10	Female	307 ± 13.8	373 ± 21.2	1.21 ± 0.03	26.2 ± 2.59	29.4 ± 2.91	1.12 ± 0.05	1.6 ± 0.80	1.0 ± 0.35
	Male	225 ± 16.5	543 ± 70.0	2.44 ± 0.34	23.5 ± 2.90	41.3 ± 3.18	1.83 ± 0.23	1.0 ± 0.0	1.4 ± 0.38
	All	266 ± 18.4	458 ± 46.7	1.83 ± 0.28	24.8 ± 1.87	35.3 ± 3.01	1.48 ± 0.17	1.3 ± 0.39	1.2 ± 0.25
75	Female	1650 ± 180	2360 ± 175	1.46 ± 0.12	141 ± 17.1	175 ± 13.5	1.27 ± 0.11	1.5 ± 0.29	1.0 ± 0.0
	Male	2070 ± 182	2820 ± 300	1.38 ± 0.15	163 ± 20.9	204 ± 18.5	1.32 ± 0.19	1.8 ± 0.25	2.5 ± 0.50
	All	1860 ± 143	2590 ± 183	1.42 ± 0.09	152 ± 13.2	190 ± 11.9	1.29 ± 0.10	1.6 ± 0.18	1.8 ± 0.37

Data are represented as the mean ± standard error (n=4 for each sex, n=8 for all)
[2.6.6.3.9 項] [2.6.7.7F 項] Nine-Month Oral Toxicity Study in Dogs (TT#[redacted]-1007)

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2.6.4.3.2.3　サル

3ヵ月間経口投与毒性試験において、MK-3102を1、3及び9 mg/kg/日でカニクイザルに13週間経口投与した際の蓄積係数（第13週／第1日）は、$AUC_{0\text{-}24\ hr}$ で1.81～2.80、C_{max} で1.73～2.32であった[表2.6.4: 8]。

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表 2.6.4: 8　　サルの 3 ヵ月毒性試験における MK-3102 の TK パラメータ：試験 13 週

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (μM·hr)			C_{max} (μM)			T_{max} (hr)	
		Day 1	Week 13	Ratio (Week 13/Day 1)	Day 1	Week 13	Ratio (Week 13/Day 1)	Day 1	Week 13
1	Female	7.59 ± 0.292	21.2 ± 1.55	2.80 ± 0.26	0.759 ± 0.0821	1.61 ± 0.221	2.15 ± 0.32	1.5 ± 0.50	1.2 ± 0.44
	Male	7.15 ± 0.252	17.8 ± 0.929	2.50 ± 0.18	0.657 ± 0.0402	1.43 ± 0.206	2.21 ± 0.39	1.5 ± 0.50	1.5 ± 0.50
	All	7.37 ± 0.198	19.5 ± 1.11	2.65 ± 0.16	0.708 ± 0.0468	1.52 ± 0.141	2.18 ± 0.23	1.5 ± 0.32	1.3 ± 0.31
3	Female	18.9 ± 0.901	54.3 ± 3.54	2.90 ± 0.28	1.85 ± 0.314	4.15 ± 0.272	2.32 ± 0.23	2.0 ± 1.0	1.5 ± 0.50
	Male	26.5 ± 1.00	63.6 ± 2.31	2.40 ± 0.06	1.94 ± 0.260	4.03 ± 0.406	2.10 ± 0.07	2.7 ± 0.67	2.7 ± 0.67
	All	22.7 ± 1.82	58.9 ± 2.81	2.65 ± 0.17	1.89 ± 0.183	4.09 ± 0.220	2.21 ± 0.12	2.3 ± 0.56	2.1 ± 0.45
9	Female	67.1 ± 11.8	156 ± 12.2	2.43 ± 0.35	7.15 ± 1.59	12.6 ± 0.772	1.93 ± 0.39	1.3 ± 0.33	1.3 ± 0.33
	Male	88.1 ± 6.12	161 ± 21.9	1.81 ± 0.17	6.72 ± 1.25	11.4 ± 2.29	1.73 ± 0.33	2.0 ± 1.0	2.0 ± 0.0
	All	77.6 ± 7.59	158 ± 11.3	2.12 ± 0.22	6.94 ± 0.911	12.0 ± 1.11	1.83 ± 0.23	1.7 ± 0.49	1.7 ± 0.21

Data are represented as the mean ± standard error (n=3 for each sex, n=6 for all)
[2.6.6.3.11 項] [2.6.7.7G 項] Three-Month Oral Toxicity Study in Cynomolgus Monkeys (TT#[redacted]-6010)

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2.6.4.4　分布

2.6.4.4.1　ラットにおける組織分布

0.5%メチルセルロースに懸濁したMK-3102を2 mg/kgで単回経口投与後、定量的全身オートラジオグラフィー法により、雄 Long-Evans（LE）ラットの[^{14}C]-MK-3102由来の放射能の組織分布を評価した[表2.6.4: 9]。試験を通じて、組織中の放射能量は経時的に減少し、終了前数回の試料採取時点では数%となった（投与72時間後の時点で20%であったのが、168、336及び672時間後には、それぞれ3.91%、2.04%及び1.48%となった）ことから、ラットでは組織に残留しないことが示唆されている[2.6.5.4 項]。

投与1時間後には、血漿やほとんどの組織で[^{14}C]-MK-3102由来の放射能が最高濃度に到達しており、吸収及び組織分布が速やかであることを示している。消化管内容物、胆汁及び尿以外で放射能の C_{max} が最も高い組織は、ブドウ膜、ハーダー腺、眼球、下垂体及び腎髄質で、7.42～112 µg eq/gであった。ブドウ膜、有色皮膚及び髄膜では、[^{14}C]-MK-3102由来の放射能濃度が投与24時間後に最高となり、その後緩徐に低下して、投与672時間後の最終試料採取時までに濃度が最低となった。このようなメラニン含有組織中の放射能濃度-時間プロファイルは、血液や血漿に比して消失が緩徐であった。また、有色皮膚では非有色皮膚よりも[^{14}C]-MK-3102由来の放射能濃度が高かった。以上のデータから、[^{14}C]-MK-3102由来の放射能がメラニンを含有する有色組織に親和性を示すことが明らかとなり、この結合は可逆的と考えられた。MK-3102は、290～700 nmでの吸光度が低く、モル吸光係数（MEC）を算出すると閾値（1000 L mol^{-1} cm^{-1}）を下回るため、光毒性試験を実施する必要はないと考える[2.6.6.8.7.5 項]。

また、血液中の[^{14}C]-MK-3102由来の放射能の消失半減期が約86時間であったのに対して、腎、肝、唾液腺、脾臓、肺などの DPP-4活性の高い組織では約140～220時間であり、長く留まっていた。

脳室周囲以外の中枢神経系組織では投与後72時間、精巣では168時間まで放射能が検出され、[^{14}C]-MK-3102由来の放射能が血液-脳関門及び血液-精巣関門を通過したことを示している。

液体シンチレーション計数法に基づく放射能の血液／血漿中濃度比の平均値は、投与後4時間の時点の0.5から投与後168時間の時点の0.8までの範囲を示し、in vitroで測定したラット血液／血漿中濃度比と一致している [2.6.4.4.4 項]。

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表 2.6.4: 9　　[^{14}C]-MK-3102（2 mg/kg）経口投与後の Long-Evans ラットの組織中放射能濃度 [a]

	[^{14}C]-MK-3102 ng 当量/g							
	測定時点							
組織	1時間	4時間	8時間	24時間	72時間	168時間	336時間	672時間
副腎	4950	2640	2170	662	159	75.2	24.0	14.1
動脈壁	3350	1230	1050	275	82.6	30.1	ND	ND
胆汁	6070	3230	ND	ND	ND	ND	ND	ND
血液	1260	558	371	62.7	12.4	BLQ	BLQ	ND
骨	129	78.3	72.8	BLQ	ND	ND	ND	ND
骨髄	3160	1360	1220	408	127	68.4	21.1	10.2
小脳	784	316	246	50.5	10.4	BLQ	ND	ND
大脳	862	345	285	51.8	9.28	BLQ	ND	ND
脳髄質	833	303	264	43.4	BLQ	BLQ	ND	ND
嗅脳	699	251	221	69.6	12.1	BLQ	ND	ND
頬腺	4060	1590	978	173	20.9	ND	ND	ND
尿道球腺	2180	1120	823	177	42.7	ND	ND	ND
盲腸	1890	828	1610	251	44.2	25.7	ND	ND
盲腸内容物	1320	2380	15400	690	67.5	15.5	BLQ	ND
食道内容物	5590	2090	1500	388	39.0	BLQ	ND	ND
大腸内容物	934	NR	25500	1570	117	20.5	BLQ	ND
小腸内容物	37300	56400[b]	2560	454	80.3	14.9	BLQ	BLQ
胃内容物	30900	9120	1900	167	16.2	BLQ	BLQ	BLQ
横隔膜	1640	837	562	89.1	31.3	BLQ	ND	ND

[a]　測定時点あたりラット1例
BLQ　定量限界未満（<9.25 ng [^{14}C]-MK-3102当量/g）
ND　検出不能（背景組織や周辺組織と試料が識別できなかった）
NR　確認不能（切片に組織が確認できなかった）
[b]　この数値を算出するため使用した同組織の試料2つが定量上限を上回っていた（>90800 ng [^{14}C]-MK-3102当量/g）

[2.6.5.4 項]

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表 2.6.4: 9　　[^{14}C]-MK-3102（2 mg/kg）経口投与後の Long-Evans ラットの組織中放射能濃度 [a]（続き）

	[^{14}C]-MK-3102 ng 当量/g							
	測定時点							
組織	1時間	4時間	8時間	24時間	72時間	168時間	336時間	672時間
精巣上体	1780	904	606	284	54.5	44.7	15.4	BLQ
食道	1800	754	531	83.7	34.3	9.62	ND	ND
眼窩外涙腺	2900	1430	1110	351	93.4	59.1	18.6	16.6
水晶体	74.8	364	669	2000	325	258	65.7	11.7
ブドウ膜	35500	43800	72200	112000[b]	53200	41100	31400	24500
硝子体液	1250	2090	2530	3370	1040	1250	260	192
眼球	6290	9680	14300	21500	11500	11100	5720	4440
筋膜	1630	669	455	102	36.0	23.8	10.4	ND
脂肪（腹部）	125	154	81.1	53.1	11.6	ND	ND	ND
脂肪（褐色）	2990	1050	425	100	27.9	13.5	13.7	13.1
ハーダー腺	18000	15600	26800	2750	649	79.6	26.0	ND
眼窩内涙腺	2900	1460	909	406	102	78.7	20.6	17.6
腎皮質	6710	3460	3270	2570	1520	1030	292	97.0
腎髄質	7420	3770	3330	2800	1930	1600	406	132
腎臓	7030	3590	3240	2760	1690	1280	351	112
大腸	2330	1490	907	255	50.1	16.2	ND	ND
肝臓	6120	3140	1890	616	256	90.0	30.5	10.4
肺	4130	1710	1410	397	262	97.8	31.7	11.8
リンパ節	2340	1370	896	305	80.6	39.3	24.5	ND

[a]　測定時点あたりラット1例
BLQ　定量限界未満（<9.25 ng[^{14}C]-MK-3102当量/g）
ND　検出不能（背景組織や周辺組織と試料が識別できなかった）
[b]　この数値を算出するため使用した同組織の試料2つが定量上限を上回っていた（>90800 ng[^{14}C]-MK-3102当量/g）

[2.6.5.4 項]

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表 2.6.4: 9　　[^{14}C]-MK-3102（2 mg/kg）経口投与後の Long-Evans ラットの組織中放射能濃度 [a]（続き）

	[^{14}C]-MK-3102 ng 当量/g							
	測定時点							
組織	1時間	4時間	8時間	24時間	72時間	168時間	336時間	672時間
髄膜	3720	1400	2760	2020	490	1220	620	584
筋組織	1110	474	377	60.7	11.9	ND	ND	ND
心筋	1900	878	574	149	43.6	13.1	ND	ND
鼻甲介	1980	858	702	260	38.1	ND	ND	ND
膵臓	2770	1220	751	205	47.5	15.6	BLQ	ND
下垂体	9680	2620	3920	757	165	57.6	11.9	10.2
包皮腺	1530	1380	1490	535	194	19.2	BLQ	ND
前立腺	3330	2340	1540	264	41.3	BLQ	BLQ	ND
唾液腺	4570	2490	1320	333	85.5	43.7	14.2	12.3
耳下唾液腺	2590	1370	978	180	44.5	25.3	13.8	13.2
精嚢	1730	951	652	132	19.8	BLQ	ND	ND
皮膚（非有色）	1060	592	367	83.9	28.5	BLQ	ND	ND
皮膚（有色）	2810	3310	5540	6120	2600	101	23.2	21.1
小腸	2940	1800	999	383	77.6	32.7	21.2	BLQ
脊髄	826	326	266	49.5	9.73	ND	ND	ND
脾臓	3570	2050	1430	448	219	118	38.8	24.7
胃	5010	1380	763	208	37.3	ND	ND	ND
精巣	1340	988	762	244	37.5	10.7	BLQ	ND
胸腺	2370	1190	921	285	153	67.1	18.8	10.5
甲状腺	2450	1130	344	190	47.9	40.0	23.0	16.1
膀胱	2130	954	550	281	34.0	BLQ	ND	ND
尿	46500	2780	2810	294	54.2	15.2	ND	ND

[a]　測定時点あたりラット1例
BLQ　定量限界未満（<9.25 ng[^{14}C]-MK-3102当量/g）
ND　検出不能（背景組織や周辺組織と試料が識別できなかった）

[2.6.5.4 項]

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2.6.4.4.2　ラット及びウサギにおける胎盤通過

妊娠 Sprague Dawley ラットに0.5%メチルセルロースで懸濁した MK-3102 10 mg/kg 又は100 mg/kg を妊娠6～20日に1日1回経口投与し、胎盤通過を検討した[2.6.5.5 項]。妊娠20日の最終投与2時間後及び24時間後の時点で、母動物と胎児の血液試料を採取し、血漿中 MK-3102濃度を測定した。試験終了日まで全動物が生存した。[表2.6.4: 10]に示したとおり、試験対象とした2群では2回の測定時点で MK-3102血漿中濃度の胎児／母体比が0.52～0.67となったことから、MK-3102がラットの胎盤を通過する可能性が示唆された。

表 2.6.4: 10　1 日 1 回経口投与を実施したラットの妊娠 20 日の MK-3102 平均血漿中濃度

動物種	用量 (mg/kg/day)	時間 (hr)	血漿中濃度 (μM)[a]		比[b]
			母体	胎児	（胎児／母体）
ラット	10	2	25.3 ± 2.68	14.2 ± 1.13	0.571 ± 0.0397
		24	8.00 ± 1.68	4.07 ± 0.665	0.522 ± 0.0246
	100	2	164 ± 9.59	101 ± 2.22	0.618 ± 0.0267
		24	57.7 ± 6.74	37.3 ± 2.66	0.672 ± 0.0859

[a]　平均値±標準誤差（測定時点あたり8例）
[b]　数値は個々の胎児／母体血漿比の平均値±標準誤差

[2.6.5.5 項]

ほかにも、妊娠 Dutch Belted ウサギを用いて、妊娠20日の母体と胎児の血漿中濃度を測定することにより、MK-3102の胎盤通過を検討した。妊娠7～20日に1日1回0.5%メチルセルロースで懸濁した MK-3102 50 mg/kg を経口投与した。妊娠20日の最終投与2時間後及び24時間後の時点で、母体と胎児の血液試料を採取した。試験終了予定日まで全動物が生存した。[表2.6.4: 11]に示したとおり、2回の測定時点で MK-3102血漿中濃度の胎児／母体比が0.55～0.57となった。上記のデータから、ラットと同じく MK-3102がウサギ胎盤を通過する可能性が示唆された。

表 2.6.4: 11　1 日 1 回経口投与を実施したウサギの妊娠 20 日の MK-3102 平均血漿中濃度

動物種	用量 (mg/kg/day)	時間 (hr)	血漿中濃度 (μM)[a]		比[b]
			母体	胎児	（胎児／母体）
ウサギ	50	2	85.3 ± 2.62	47.0 ± 3.62	0.550 ± 0.0264
		24	23.2 ± 5.12	13.4 ± 3.07	0.572 ± 0.0154

[a]　平均値±標準誤差（測定時点あたり8例）
[b]　数値は個々の胎児／母体血漿比の平均値±標準誤差

[2.6.5.5 項]

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2.6.4.4.3　In vitro 血漿蛋白結合

平衡透析法を用いて、[^{3}H]-MK-3102の血漿蛋白に対する可逆的結合を測定した[2.6.5.6 項]。MK-3102は血漿蛋白との結合率は高くなく、種差が認められた。CD1マウス、Sprague Dawley ラット、ビーグル犬、アカゲザル、カニクイザル、雌 Dutch Belted ウサギ及びヒト血漿中の[^{3}H]-MK-3102（1、10、100 μM）の平均非結合型分率は、それぞれ38、15、43、85、87、78及び68%であった。1～100 μM の濃度では、非結合型分率に顕著な変化は認められなかった[表2.6.4: 12]。

臨床相当濃度（1～1000 nM）で、ヒト血漿を用いて実施した追加の in vitro 試験では、[^{3}H]-MK-3102が濃度依存的にヒト血漿蛋白と結合し、非結合型分率は1 nM では約25%、1000 nM では約76%であった[表2.6.4: 13]。この濃度依存的な血漿蛋白結合は、10 nM 未満で顕著であったことから、用量が5 mg 未満の場合ではヒトでの薬物動態が用量比例性を示さないと考えられる。用量が10 mg を超え、平均濃度が50 nM を超えると、薬物動態プロファイルの大半の非結合型分率は一定に近づき、これは10～100 mg の用量範囲でヒトでの薬物動態に用量比例性がみられたことと一致する[2.7.2.1.1 項]。濃度依存的な蛋白結合がリナグリプチンにも同様に認められたことから、血漿 DPP-4との結合が飽和するためであると考えられている[資料4.3: 201]。さらに、DPP-4 ノックアウトマウス血漿にヒト遺伝子組換え DPP-4を6 nM（報告されているヒト血漿中 DPP-4濃度）[資料4.3: 201]添加し in vitro 蛋白結合試験を実施した。予想どおり、ノックアウトマウス血漿では MK-3102は濃度依存的な蛋白結合率の変化を示さなかったが、ヒト DPP-4を添加したノックアウトマウス血漿では濃度依存性を示し、DPP-4への結合が飽和するという仮説が支持された。[表2.6.4: 14]。

このほか、障害の程度が異なる腎機能障害者（軽度、中等度、重度、血液透析を必要とする末期腎不全）及び対照として健康被験者の投与前血漿試料を用いて、in vitro 血漿蛋白結合を測定した（009試験）[2.7.2.2.3.3 項]。腎機能障害者と対照健康被験者の間で、MK-3102（1～1000 nM）の血漿蛋白結合にほとんど差がみられず、腎機能障害の影響はないことが示された[2.6.5.6 項][2.7.2.2.3.3 項]。同じく、肝機能障害者でも MK-3102の血漿蛋白結合に差は認められなかった（031試験）[2.6.5.6 項][2.7.2.2.3.4 項]。

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表 2.6.4: 12　マウス、ラット、ウサギ、イヌ、サル及びヒトにおける MK-3102 の in vitro 血漿蛋白結合

濃度	血漿中の非結合型分率（%）[a]						
	CD1 マウス	ラット	ウサギ	イヌ	アカゲザル	カニクイザル	ヒト
1 μM	36[b]	14±0.5	80±3.6[e]	41[b]	84[c]	85±3.6[e]	66±2.6
10 μM	38±0.5	15±0.9	76±0.8[d]	42±1.5	86±2.1	87±1.9[e]	68±0.8
100 μM	38[c]	16±0.3	77±15[e]	46±0.4	86±0.4	88±1.3[e]	71±2.6
平均[f]	38	15	78	43	85	87	68

[a] 数値は3回の測定の平均値±SD
[b] 2回の反復測定による平均値
[c] 1回の測定による数値
[d] 数値は4回の測定の平均値±SD
[e] 数値は5回の測定の平均値±SD
[f] 3通りの濃度から得られた非結合型分率の平均値

[2.6.5.6 項]

表 2.6.4: 13　ヒトにおける[^{3}H]-MK-3102 の濃度別の in vitro 血漿蛋白結合

濃度 (nM)	ヒト血漿中の非結合型分率（%）[a]
1	25 ± 2.5
5	36 ± 1.0
10	43 ± 2.6
25	56 ± 0.4
50	64 ± 3.3
100	71 ± 1.3
250	76 ± 2.8
1000	76 ± 2.5

[a] 数値は5回の測定の平均値±SD

[2.6.5.6 項]

表 2.6.4: 14　ヒト組換え DPP-4 添加及び非添加の DPP-4 ノックアウトマウスにおける[^{3}H]-MK-3102 の in vitro 血漿蛋白結合

濃度 (nM)	DPP-4ノックアウトマウス血漿中の非結合型分率（%）[a]	
	ヒト DPP-4非添加	ヒト DPP-4添加[b]
1	32 ± 0.6	23 ± 2.1
5	38 ± 2.1	24 ± 1.9
10	37 ± 2.4	26 ± 1.2
25	38 ± 2.6	28 ± 0.7
50	41 ± 0.5	34 ± 1.0
100	41 ± 1.2	36 ± 0.6
250	40 ± 0.1	41 ±0.8
1000	39 ± 0.6	42 ± 1.0

[a] 数値は3回の測定の平均値±SD
[b] ヒト DPP-4の最終濃度は6 nM であった。

[2.6.5.6 項]

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2.6.4.4.4　In vitro 血液／血漿比

MK-3102と新鮮全血をインキュベートして、MK-3102（1～10 μM）の血球移行性を測定した。血液／血漿中濃度比は本試験で設定したMK-3102濃度では濃度依存性はみられず、ラット、イヌ、サル及びヒトでは平均値が、それぞれ0.6、0.9、1.2及び0.9となった[表2.6.4: 15] [2.6.5.7 項]。以上のデータから、MK-3102が血球に移行することが示された。

表 2.6.4: 15　[^{3}H]-MK-3102 の in vitro 血液／血漿中濃度比 [a]

濃度	血液／血漿中濃度比			
	ラット	イヌ	サル	ヒト
1 μM	0.7	0.9	1.2	0.9
10 μM	0.6	0.9	1.2	0.9
平均値	0.6	0.9	1.2	0.9

[a] いずれの試験濃度も、3回の血液中濃度測定の平均値と3回の血漿中濃度測定の平均値を用いて血液／血漿比を得た。

[2.6.5.7 項]

2.6.4.4.5　P-糖蛋白を介した輸送

ヒト及びマウスの P-糖蛋白（P-gp）をコードしたヒト MDR1及びマウス Mdr1a を発現した LLC-PK1細胞を用いて、MK-3102の双方向輸送を評価した。LLC-PK1対照細胞では、MK-3102の受動的膜透過性が高かった（P_{app}: 26 × 10^{-6} cm/s）。LLC-MDR1発現細胞では双方向の輸送比（B-A/A-B 比）が1.0～1.2であり、MK-3102はヒト MDR1に対する基質ではないことを示している[表2.6.4: 16]。LLC-Mdr1a 発現細胞の B-A/A-B 比は2.4～2.7であり、MK-3102がマウス Mdr1a の弱い基質であることが示唆された。陽性対照として用いたベラパミルでは、LLC-PK1、LLC-MDR1及び LLC-Mdr1a の各細胞の B-A/A-B 比がそれぞれ1.3、4.1及び7.1となり、各細胞株の機能性が確認された[2.6.5.8 項]。このデータに基づけば、MK-3102はヒトの中枢神経系へ移行するものと予想される。

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表 2.6.4: 16　LLC-MDR1（ヒト P-gp）細胞株と LLC-Mdr1a（マウス P-gp）細胞株における MK-3102 の P-gp 基質認識性の評価

化合物	濃度 (μM)	P_{app} (B-A)/P_{app} (A-B) 比 (N=1)			P_{app} (x 10^{-6} cm/s) (N=1) LLC-PK1
		LLC-PK1[a]	LLC-MDR1	LLC-Mdr1a	
MK-3102	0.1	0.9	1.2	2.7	24
MK-3102	0.5	1.0	1.1	2.4	27
MK-3102	1	1.0	1.0	2.4	27
ベラパミル	5	1.3	4.1	7.1	31

[a]　対照細胞

[2.6.5.8 項]

2.6.4.5　代謝

2.6.4.5.1　ラットの in vivo 代謝

Sprague Dawley ラットに胆管カニュレーションを実施し、[^{3}H]-MK-3102 [図2.6.4: 1] 2 mg/kg を経口投与して代謝を検討した[2.6.5.9 項]。尿、胆汁、糞中の平均排泄率は、それぞれ55%、17%及び5.0%であった[2.6.4.6.1 項]。MK-3102の主要消失経路は、腎排泄であり（総放射能排泄量の約61%）、代謝（主に酸化）は副次的な消失経路であった（総放射能排泄量の約28%）。ラット尿では、主な放射性成分は未変化体であり、尿中放射能量の86%を占めていた。尿中に検出された代謝物はいずれも酸化代謝物であり、ピロロピラゾールテトラヒドロピラン部位に18 Da が付加された代謝物（M1）、開環カルボン酸体（M2）、ジヒドロキシル化体（M3）、モノヒドロキシル化体（M5）及びピロロピラゾール部位に18 Da が付加された代謝物（M6）が含まれていた[図2.6.4: 2][表2.6.4: 17]。ラット胆汁では、M2及び M3が主な代謝物であり、これ以外に総放射能排泄量の2%未満の未知の放射能ピークが検出された[図2.6.4: 2][表2.6.4: 17]。

[^{3}H]-MK-3102 20 mg/kg を投与した無処置ラットの血漿中の主な放射性成分は未変化体であった（投与2、6及び24時間後の時点で血中総放射能量の99%超）。ラット血漿で検出された微量代謝物は M1、M2及び M3であった（血中総放射能量の1%未満）[図2.6.4: 3]。

2.6.4.5.2　イヌの in vivo 代謝

雄ビーグル犬に胆管カニュレーションを実施し、[^{14}C]-MK-3102 [図2.6.4: 1]2 mg/kg を経口投与してイヌでの代謝を検討した[2.6.5.9 項]。尿、胆汁及び糞中の平均排泄率は、それぞれ60%、5.1%及び7.0%であった[2.6.4.6.2 項]。イヌ尿では、未変化体が唯一検出された放射性成分であり、尿中放射能量の100%を占めていたことから、イヌでは腎排泄が主要消失経路であることが明らかになった（総放射能排泄量の約82%）。イヌ胆汁では、未変化体が胆汁中放射能量の約92%であり、総放射能排泄量の約6.4%を占めていた。放射能ピークのうち1つが同定できなかった（総放射能排泄量の1%未満）。

同胆管カニュレーションされたイヌから血漿試料を採取し、血漿中代謝物のプロファイリング

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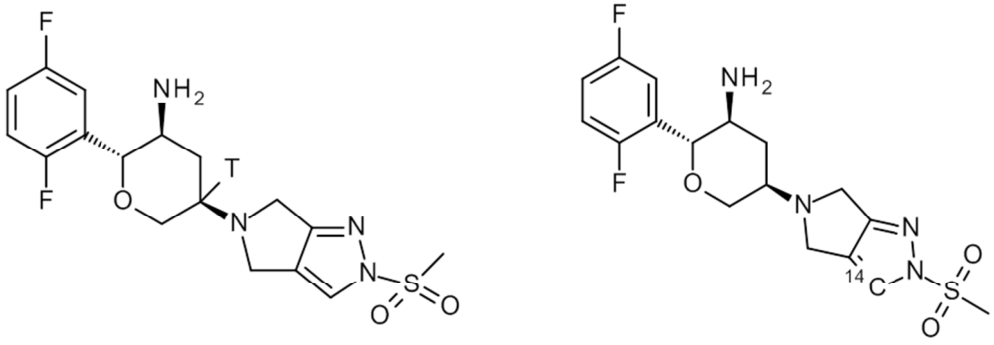
を検討した。投与2、6及び24時間後の時点では、MK-3102が主な血漿中放射性成分であった（血漿中放射能量の87%超）。また、イヌ血漿中に微量のスルホンアミド加水分解物（M4）が検出された（血漿中放射能量の13%未満）[図2.6.4: 2] [図2.6.4: 3] [表2.6.4: 17]。

2.6.4.5.3　ヒトの in vivo 代謝

健康男性被験者6名に[^{14}C]-MK-3102[図2.6.4: 1]25 mg（約2.5 μCi）内用液剤を単回投与し、代謝及び排泄を検討した[2.6.5.9 項]。投与後20日まで様々な間隔で血漿、尿及び糞を採取した。排泄物中の総放射能排泄率の平均値は約78%で、尿及び糞中の排泄率はそれぞれ74.4%及び3.4%であった[2.6.5.15 項]。動物と同様に、MK-3102は主に腎排泄により消失し、代謝の寄与はわずかであった。この結果は、日本人での第 I 相反復投与試験（005試験）で、定常状態での投与量の約74%が尿中に未変化体として排泄された尿中薬物動態の結果と一貫した結果であった[2.7.2.2.1.1 項]。尿中に排泄された放射能の大半（約89%）が未変化体であった。未変化体以外の尿中放射能は、M1、M3、M5及び M6など数種の酸化代謝物であり、いずれも尿中総放射能量の3%未満であった。これらの代謝物は、ラットでも検出された[図2.6.4: 2] [表2.6.4: 17]。糞中に排泄された放射能がわずかであるため、糞中の代謝物プロファイリングは実施しなかった。

ヒト血漿では、未変化体が血漿中放射能のほぼ全量を占め、主な血漿中代謝物は検出されなかった[図2.6.4: 3]。



T はトリチウム標識した部位を示す。

図 2.6.4: 1　　[^{3}H]-MK-3102 及び[^{14}C]-MK-3102 の化学構造

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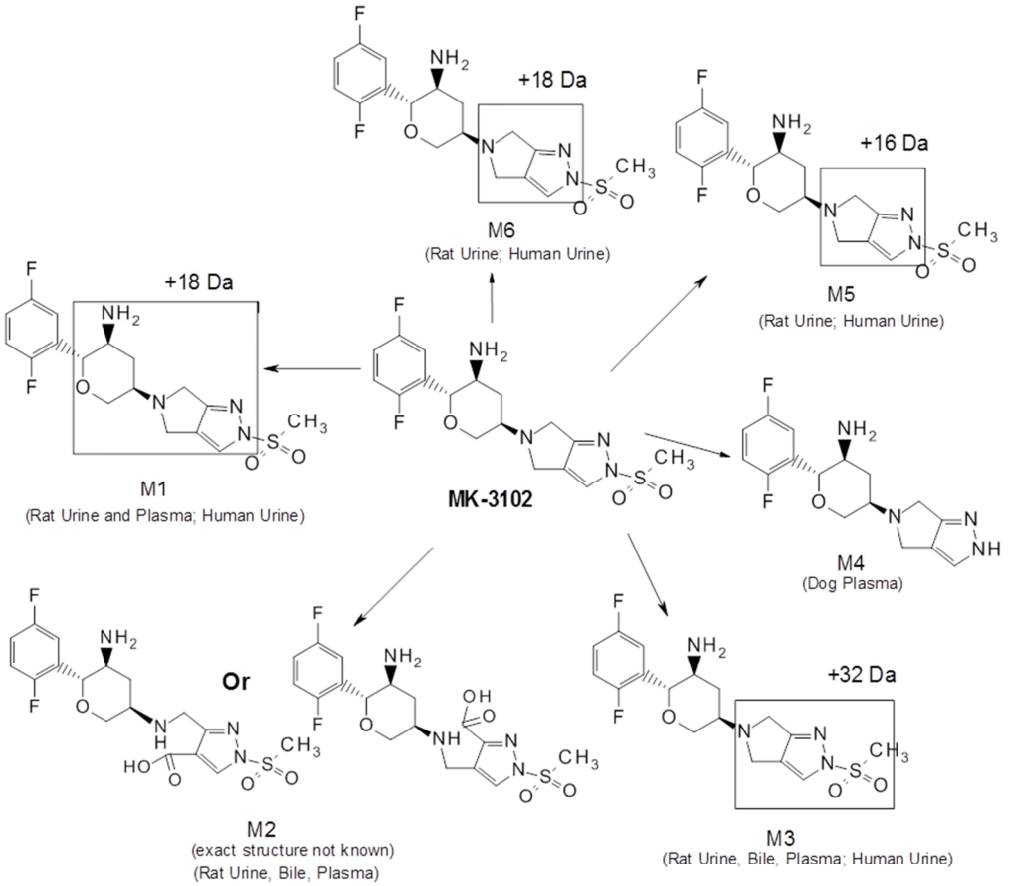

M4の合成標準物質は入手可能である。
[2.6.5.10 項]

図 2.6.4: 2　　ラット、イヌ及びヒトの MK-3102 推定代謝物

表 2.6.4: 17　非臨床試験で用いた動物種及びヒトに MK-3102 経口投与後の血漿中及び排泄物中の MK-3102 及びその代謝物の有無

動物種	ラット				イヌ				ヒト		
生体試料	血漿	胆汁	尿	糞	血漿	胆汁	尿	糞	血漿	尿	糞
MK-3102	X	X	X	N.A.	X	X	X	N.A.	X	X	N.A.
M1	X	-	X	N.A.	-	-	-	N.A.	-	X	N.A.
M2	X	X	X	N.A.	-	-	-	N.A.	-	-	N.A.
M3	X	X	X	N.A.	-	-	-	N.A.	-	X	N.A.
M4	-	-	-	N.A.	X	-	-	N.A.	-	-	N.A.
M5	-	-	X	N.A.	-	-	-	N.A.	-	X	N.A.
M6	-	-	X	N.A.	-	-	-	N.A.	-	X	N.A.
X：LC/MS 及びラジオクロマトグラフィーにより検出 -：検出せず N.A.：解析未実施（回収された放射能がわずかであったため）											

[2.6.5.9 項]

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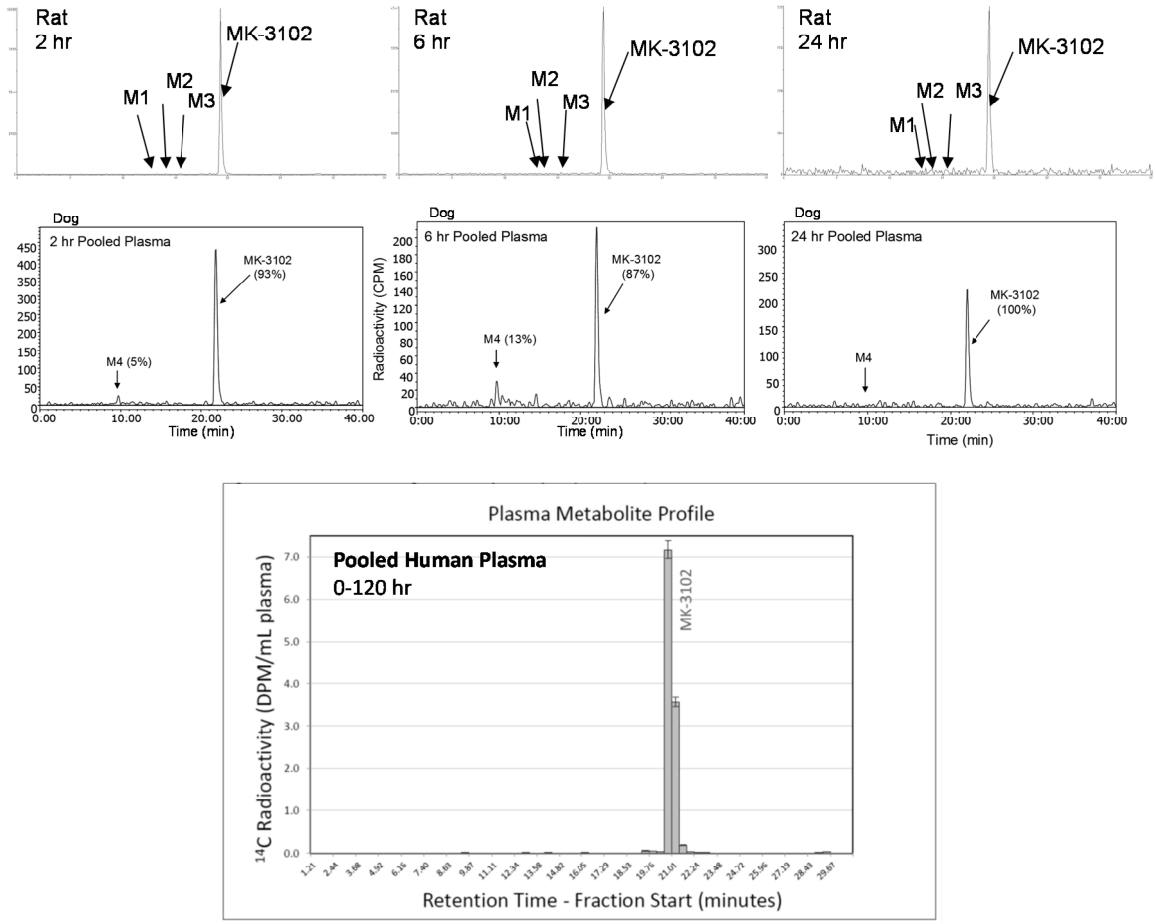

[2.6.5.9 項]

図 2.6.4: 3　[^{3}H]-MK-3102 又は[^{14}C]-MK-3102 を経口投与したラット（2 mg/kg）、イヌ（2 mg/kg）及びヒト（25 mg）の血漿中代謝物の代表的 HPLC-ラジオクロマトグラム

2.6.4.5.4　In vitro 代謝

ラット、イヌ、サル及びヒトの肝細胞とともに2時間インキュベーションした際の MK-3102の in vitro 代謝を検討した。また、NADPH 存在下で CD1マウス、ラット、イヌ、サル、雌 Dutch Belted ウサギ及びヒトの肝ミクロソームとともに1時間インキュベーションした際の MK-3102の in vitro 代謝についても検討した。いずれの肝調製画分を用いた in vitro 試験系でも、MK-3102の代謝はみられなかった[2.6.5.11 項]。このことは、肝代謝がわずかであったという in vivo での結果と一貫していた。

2.6.4.6　排泄

2.6.4.6.1　ラット

雄 BDC Sprague Dawley ラットにおいて2 mg/kg 経口投与後の[^{3}H]-MK-3102の排泄を検討した。

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本検討では投与120時間後まで排泄物を採取した。経口投与後、放射能はほとんど尿中に排泄され（55%）、胆汁や糞中に排泄される割合は小さかった（それぞれ17%及び5%）[表2.6.4: 18][2.6.5.13 項]。この試験の総放射能排泄率は77%であった。これは、ラット QWBA 試験で投与72時間後に投与量の約20%が組織中に検出されたが、最終試料採取時には組織中の放射能がごくわずかになっていた結果（投与672時間後で投与量の1.48%）とおおむね一貫した結果であり、ラット組織に対して不可逆的結合を示さないことが明らかになった[2.6.4.4.1 項] [2.6.5.4 項]。

ラットでは、非結合型 MK-3102の腎クリアランス（4.5 mL/min/kg）が糸球体ろ過量の約1/2であり（約10～12 mL/min/kg）[資料4.3: 202]、腎排泄過程での再吸収を示唆している。

表 2.6.4: 18　[^{3}H]-MK-3102 又は[^{14}C]-MK-3102 単回経口投与後のラット及びイヌでの放射能排泄 [a]

用量 (mg/kg)	投与経路	投与量に対する排泄された放射能の割合				
		尿	胆汁	糞	ケージ洗浄液	計
ラット（n=4）						
2	経口	55 ± 4.0	17 ± 2.8	5.0 ± 1.7	ND	77 ± 0.4
イヌ（n=3）						
2	経口	60 ± 4.5	5.1 ± 1.3	7.0 ± 1.1	1.5 ± 0.2	73 ± 4.7

[a] 数値はラット4例又はイヌ3例の平均値±SD
ND：検出せず

[2.6.5.13 項][2.6.5.14 項]

2.6.4.6.2　イヌ

雄胆管カニュレーションビーグル犬に[^{14}C]-MK-3102（2 mg/kg）を経口投与したところ、放射能のほとんどが尿中に排泄され（60%）、一部が胆汁、糞及びケージ洗浄液（それぞれ5.1%、7.0%及び1.5%）に排泄された[表2.6.4: 18][2.6.5.14 項]。試料採取期間は、胆汁が0～72時間、尿、糞及びケージ洗浄液が0～120時間であった。このデータにより、イヌでは腎排泄が放射能消失の主要経路であることが示された。

イヌの非結合型 MK-3102の腎クリアランスを算出したところ1.7 mL/min/kg と糸球体ろ過量の1/2をわずかに下回り（約4～6 mL/min/kg）[資料4.3: 203] [資料4.3: 204]、ラットと同様、腎排泄過程での再吸収が示唆された。

2.6.4.6.3　ラットにおける乳汁中移行

妊娠6日から授乳14日の間、MK-3102を2 mg/kg/day 又は10 mg/kg/day で連日経口投与し、授乳14日に母ラットの血漿及び乳汁中の MK-3102濃度を測定することにより、授乳期ラットの MK-3102の乳汁中移行を検討した[2.6.5.16 項]。授乳14日の最終投与2時間後に眼窩洞より血液試料を採取し、採血後速やかにオキシトシンを筋肉内投与した。約5分後から10分間かけて乳汁を吸引採取し

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た。2 mg/kg/day 及び10 mg/kg/day 投与後、乳汁中の MK-3102濃度は母動物の血漿中濃度のそれぞれ約76%及び81%となり、授乳期ラットの乳汁中に血中の MK-3102が移行することが示された。

2.6.4.7　薬物動態学的薬物相互作用

In vitro データにより、MK-3102に薬物相互作用が生じる可能性が低いことが示された。詳細なデータを以下に示す。

2.6.4.7.1　チトクロム P450の基質としての MK-3102の評価

MK-3102（1 μM）を発現系 CYP 分子種 CYP3A4、2D6、2C8、2C9、2C19、1A2、2A6、2B6及び2E1とインキュベートしたところ、代謝反応はみられなかった。したがって、CYP 酵素に対する特異的阻害抗体を用いた試験を実施することはできなかった[2.6.5.12 項]。

2.6.4.7.2　ヒト肝チトクロム P450、UGT1A1及び SULT1E1の阻害

ヒト肝ミクロソームを用いてプレインキュベーションは実施せずに、CYP1A2、2B6、2C8、2C9、2C19、2D6及び3A4に対する MK-3102の阻害能を評価した。MK-3102は、主要な CYP に対する可逆的阻害剤ではなく、IC_{50}値が100 μM超であった [2.6.5.17 項]。また、MK-3102 10 μM及び50 μMで CYP3A4の時間依存的な阻害はみられなかった[2.6.5.18 項]。

CYP3A4及び CYP2C9以外にも、UGT1A1及び SULT1E1の2種類はエチニルエストラジオール代謝に関与する酵素である。MK-3102が経口避妊薬（エチニルエストラジオール）の薬物動態に及ぼす影響を検討した。この in vitro 試験では MK-3102がヒト肝ミクロソームの UGT1A1を介したエストラジオール3-グルクロン酸抱合及びヒト肝サイトソールの SULT1E1を介したエチニルエストラジオール3-硫酸化に及ぼす阻害作用を評価した。この結果により、MK-3102は UGT1A1及び SULT1E1の阻害剤ではないことが示された（IC_{50} 100 μM 超）[2.6.5.20 項]。

2.6.4.7.3　チトクロム P450酵素の誘導

ヒト初代肝細胞を用いて MK-3102の CYP3A4、1A2及び2B6に対する誘導能を評価した。ドナー3例から採取し、平板培養のうえ凍結保存したヒト肝細胞を0.1～20 μM の MK-3102とともに48時間インキュベーションした。CYP3A4、1A2及び2B6の mRNA 及び酵素活性を測定し、溶媒（DMSO）で処理した肝細胞と数値を比較した。CYP3A4、1A2及び2B6誘導の陽性対照として、それぞれリファンピシン（10 μM）、オメプラゾール（50 μM）及びフェノバルビタール（1000 μM）を用いた。MK-3102による CYP3A4、1A2及び2B6の mRNA 及び酵素活性の誘導はごくわずかで、陽性対照の誘導作用の10%に満たなかった。以上のデータにより、in vitro で評価した濃度では MK-3102が CYP3A4、CYP1A2及び CYP2B6の誘導剤ではないことが示された[2.6.5.19 項]。

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2.6.4.7.4　ヒト P-gp、BCRP、MATE1、OATP1B1及び OATP1B3の阻害

[^{3}H]ジゴキシン（0.1 μM）を基質に、ヒト MDR1を形質導入した LLC-PK1細胞を用いて、MK-3102がヒト MDR1 P-糖蛋白（P-gp）に及ぼす影響を評価した。試験した濃度範囲（0.3～300 μM）では、MK-3102が LLC-MDR1細胞のヒト P-gp を介したジゴキシン輸送を著しく阻害しなかったことから（正味の輸送の低下は20%未満）、MK-3102はヒト P-gp の阻害剤ではないことが示唆された [2.6.5.21 項]。

ほかにも、ヒトトランスポーター乳癌耐性蛋白（BCRP）、multidrug and toxin extrusion 1 (MATE1)、有機アニオン輸送ポリペプチド1B1及び1B3（OATP1B1及び OATP1B3）に対する MK-3102の阻害作用の有無を評価した。MK-3102（最大100 μM）は、BCRP 含有膜小胞中では BCRP を介した[^{3}H]メトトレキサートの取込みを著しく阻害しなかったため（阻害率20%未満）、ヒト排出トランスポーターBCRP の阻害剤ではないことが示された。また、MK-3102は、最高50 μM でも MATE1を介した[^{14}C]メトホルミンの取込みを著しく阻害しなかったため、MK-3102は MATE1の阻害剤ではないことが示された。さらに、MK-3102は、最高100 μM でも OATP1B1を介した[^{3}H]ピタバスタチンの取込み及び OATP1B3を介した[^{3}H]BSP の取込みを著しく阻害しなかったため、この2種類のヒト肝取込みトランスポーターの阻害剤ではないことが示された（IC_{50} 100 μM 超）[2.6.5.21 項]。

2.6.4.7.5　hOCT1、hOCT2、hOAT1、hOAT3の基質及び阻害剤としての MK-3102の評価

ヒト有機カチオントランスポーター（hOCT1及び hOCT2）又は有機アニオントランスポーター（hOAT1及び hOAT3）をコードするヒト cDNA を一過性に又は安定的に遺伝子導入したチャイニーズハムスター卵巣（CHO）-K1細胞又は MDCKII 細胞を用いて、上記トランスポーターの発現細胞への MK-3102の取込みを検討した[2.6.5.22 項]。hOCT1、hOCT2、hOAT1及び hOAT3の発現細胞への MK-3102の能動的な取込みは認められなかった。これとは対照的に、テトラエチルアンモニウム（hOCT1及び hOCT2のモデル基質）、p-アミノ馬尿酸（hOAT1のモデル基質）及びエストロン硫酸（hOAT3のモデル基質）では能動的な取込みが認められたことから、hOCT1、hOCT2、hOAT1及び hOAT3に用いられる発現系の機能性が確認された。上記のデータにより、同試験環境下では MK-3102が肝取込みトランスポーターhOCT1又は腎取込みトランスポーター（hOAT1、hOAT3及び hOCT2）の基質ではないことが示された[2.6.5.22 項]。

MK-3102は、hOAT1を介したシドホビルの取込み、hOAT3を介したシメチジンの取込み、hOCT2を介したメトホルミンの取込み、hOCT1を介したメトホルミンの取込みの強力な阻害剤ではなく、IC_{50}値が50 μM 超であった[2.6.5.22 項]。

2.6.4.8　その他の薬物動態試験

その他の薬物動態試験は実施しなかった。

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2.6.4.9　考察及び結論

MK-3102は、ラット及びイヌでは血漿クリアランスが低く、半減期が長かった。ラット及びイヌでは、吸収が速やかで絶対的経口バイオアベイラビリティが高かった（約100%）。有色Long-Evansラットに[^{14}C]-MK-3102を経口投与したところ、放射能が速やかに全身の広い範囲に分布した。[^{14}C]-MK-3102由来の放射能は、血液－脳関門及び血液－精巣関門を通過した。ラットでは、一部組織で後半の測定時点でも放射能濃度が測定可能であったが、組織中放射能は経時的に減衰し、ごく一部が確認されるにとどまったことから、組織に残留しないことが示唆された。[^{14}C]-MK-3102由来の放射能は、メラニン含有組織に親和性を示したが、この関係は可逆的なものであると考えられた。MK-3102はラット及びウサギの胎盤を容易に通過し、ラット乳汁に分泌されることが明らかになった。MK-3102の血漿蛋白結合は、非臨床試験で用いた動物種及びヒトでは低度～中等度であった。臨床相当濃度（1～1000 nM）では、ヒト血漿蛋白に対して濃度依存的な結合を示し、これはDPP-4との結合の飽和に起因するものであった。この濃度依存性は、ラットやイヌの血漿にはみられなかった。

非臨床試験で用いた動物種（ラット及びイヌ）及びヒトで、MK-3102の主要消失経路は腎排泄であった。MK-3102の放射性標識体をラット、イヌ及びヒトに経口投与した際、用量のそれぞれ55、60及び74%が尿中に排泄された。いずれの動物種でも、非結合型MK-3102の腎クリアランスは糸球体ろ過量を下回り、腎排泄過程での再吸収が示された。ラット、イヌ及びヒトの血漿中では、未変化体が主要な放射性物質であった。ヒトではMK-3102の代謝がほとんどみられず、ヒトで確認されたすべての代謝物は非臨床試験で用いた動物種においても検出された。

MK-3102の薬物相互作用リスクは概して低い。臨床相当濃度（1～1000 nM）では、MK-3102は主要なCYP分子種、UGT1A1及びSULT1E1を阻害せず、CYPを誘導しなかった。また、P-gp、BCRP、OATP1B1/1B3、hOAT1、hOAT3、hOCT1、hOCT2及びMATE1を介した輸送を阻害しない。このため、主要なCYP分子種や薬物トランスポーターの阻害及び誘導を通じた臨床的に意味のある薬物相互作用をMK-3102が引き起こす可能性は低い。臨床薬物相互作用試験[2.7.2.2.4 項]の結果は、in vitro試験の結果と一致する。MK-3102は代謝をほとんど受けず、CYP阻害剤又は誘導剤と併用しても薬物相互作用を受ける可能性は低いと考えられる。多くの外因性物質と同様に、MK-3102の膜透過性の高さから考えれば（P_{app}: 26 x 10^{-6} cm/s）、MK-3102の腎排泄過程での再吸収は受動的透過によるものである可能性が高い[資料4.3: 205]。また、MK-3102はヒトP-gp並びに取込みトランスポーターであるhOAT1、hOAT3、hOCT1及びhOCT2の基質ではない。よって、これらトランスポーターの阻害剤と併用した際、MK-3102が薬物相互作用を受ける可能性は低いと考えられる。

2.6.4.10　参考文献

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CTD 第 2 部

2.6　非臨床試験の概要文及び概要表

2.6.5　薬物動態試験概要表

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略号及び用語の定義

略号	英語	定義
AMS	Accelerator Mass Spectrometry	加速器質量分析法
AUC	Area Under the Concentration Time-Curve	濃度-時間曲線下面積
BCRP	Breast Cancer Resistance Protein	乳癌耐性蛋白
BDC	Bile Duct Cannulation	胆管カニュレーション
BSP	Sulfobromophthalein	スルホブロモフタレイン
CHO	Chinese Hamster Ovary cell	チャイニーズハムスター卵巣細胞
CL_P	Plasma Clearance	血漿クリアランス
C_{max}	Maximum Concentration	最高血漿中濃度
CNS	Central Nervous System	中枢神経系
CYP	Cytochrome P450	チトクロム P450
DDI	Drug-Drug Interaction	薬物相互作用
DPP-4	Dipeptidyl Peptidase-4	ジペプチジルペプチダーゼ-4
ES	Estron Sulfate	エストロン硫酸
ESRD	end-stage renal disease	末期腎不全
GD	Gestation Day	妊娠後 X 日
HPLC	High Performance Liquid Chromatography	高速液体クロマトグラフィー
HPLC-MS/MS	High Performance Liquid Chromatography-Tandem Mass Spectrometry	液体クロマトグラフィー／タンデム質量分析法
IC_{50}	50% Inhibitory Concentration	50%阻害濃度
IV	Intravenous	静脈内
KO	Knock-out	ノックアウト
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry	液体クロマトグラフィー／タンデム質量分析法
LD	Lactation Day	授乳期 X 日
LLC-PK	Lilly Laboratory Cells-Porcine Kidney	Lilly Laboratory Cells-Porcine Kidney
LSC	Liquid Scintillation Counter	液体シンチレーション計数器
MATE	Multidrug and Toxin Extrusion	Multidrug and Toxin Extrusion
MDCK	Madin-Darby Canine Kidney	Madin-Darby Canine Kidney
MDR / mdr	Multiple Drug Resistance Protein	多剤耐性蛋白
MEC	Molar Extinction Coefficient	モル吸光係数
mRNA	Messenger Ribonucleic Acid	伝達リボ核酸
MRT	Mean Residence Time	平均滞留時間
NADPH	β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form	（還元型）β-ニコチンアミドアデニンジヌクレオチドリン酸
N.A.	Not Analyzed	解析不能
ND	Not Determined	検出不能
OAT	Organic Anion Transporter	有機アニオントランスポーター
OATP	Organic Anion Transporting Polypeptide	有機アニオン輸送ポリペプチド
OCT	Organic Cation Transporter	有機カチオントランスポーター
PAH	p-aminohippurate	pーアミノ馬尿酸
Papp	apparent permeability coefficients	見かけの透過係数
PCR	polymerase chain reaction	ポリメラーゼ連鎖反応
P-gp	P-glycoprotein	P-糖蛋白
P.O.	Per os	経口
QWBA	Quantitative Whole Body Autoradiography	定量的全身オートラジオグラフィー
RNA	Ribonucleic acid	リボ核酸

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RT	Retention Time	保持時間
SD	Standard Deviation	標準偏差
SEM	Standard Error of Mean	標準誤差
SULT	Sulfotransferase	硫酸転移酵素
$t_{1/2}$	Half-Life	見かけの終末相半減期
TEA	Tetraethylammonium	テトラエチルアンモニウム
T_{max}	Time to the Maximum Concentration	最高濃度到達時間
UGT	Uridine Diphosphate-Glucuronosyltransferease	UDP-グルクロン酸転移酵素
Vd_{ss}	Volume of Distribution at Steady State	定常状態分布容積

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2.6.5.1　薬物動態試験：一覧表

Test Article: Omarigliptin（以下 MK-3102）

Type of Study	Test System	Method of Administration	Testing Facility [a]	Study Number	Location Volume
Absorption					
Pharmacokinetics	Rat	PO	MRL[a]	PK001	[資料 4.2.2.2: PK001]
Oral Bioavailability	Dog	IV	MRL	PK001	[資料 4.2.2.2: PK001]
Pharmacokinetics	Dog	PO	MRL	PK001	[資料 4.2.2.2: PK001]
Oral Bioavailability	Rat	IV	MRL	PK001	[資料 4.2.2.2: PK001]
Distribution					
Single-dose tissue distribution	Rat	PO	[redacted][b]	PK006	[資料 4.2.2.3: PK006]
Placental transfer[c]	Rat	PO	MRL	TT[redacted]7130	[資料 4.2.2.3: TT[redacted]7130]
Placental transfer[c]	Rabbit	PO	MRL	TT[redacted]7140	[資料 4.2.2.3: TT[redacted]7140]
Reversible plasma protein binding	Rat, Dog, Monkey, Mouse, Rabbit, Human	In vitro	MRL	PK002	[資料 4.2.2.4: PK002]
Concentration dependent reversible binding to human plasma protein	Human	In vitro	MRL	PK002	[資料 4.2.2.4: PK002]
Reversible plasma binding in DPP-4 knock-out mouse plasma with and without spiked human DPP-4	Mouse	In vitro	MRL	PK002	[資料 4.2.2.4: PK002]
Reversible binding to plasma from healthy and renal insufficient patients	Human	In vitro	MRL	PK011	[資料 4.2.2.3: PK011]
Reversible binding to plasma from healthy and hepatic insufficient patients	Human	In vitro	MRL	PK013	[資料 4.2.2.3: PK013]
Blood-to-plasma partitioning	Human, Rat, Dog, Monkey	In vitro	MRL	PK002	[資料 4.2.2.4: PK002]

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2.6.5.1　薬物動態試験：一覧表（続き）

Test Article: MK-3102

Type of Study	Test System	Method of Administration	Testing Facility [a]	Study Number	Location Volume
P-glycoprotein mediated transport and in vitro passive permeability	LLC-PK1, LLC-MDR1 (human), LLC-Mdr1a (mouse) cells	In vitro	MRL	PK002	[資料 4.2.2.4: PK002]
Metabolism					
Metabolites in bile, urine and plasma	Rat	PO	MRL	PK003	[資料 4.2.2.5: PK003]
Metabolites in urine	Rat	PO	MRL	PK009	[資料 4.2.2.4: PK009]
Metabolites in urine, bile, and plasma	Dog	PO	MRL	PK007	[資料 4.2.2.5: PK007]
Metabolites in urine, and plasma	Human	PO	MRL	PK014	[資料 4.2.2.5: PK014]
Metabolism in liver microsomes	Mouse, Rabbit, Rat, Dog, monkey, Human	In vitro	MRL	PK002	[資料 4.2.2.4: PK002]
Metabolism in hepatocytes	Rat, Dog, monkey, Human	In vitro	MRL	PK002	[資料 4.2.2.4: PK002]
Excretion					
Mass balance (urine, bile, feces)	Rat	PO	MRL	PK003	[資料 4.2.2.5: PK003]
Mass balance (urine, bile, feces)	Dog	PO	MRL	PK007	[資料 4.2.2.5: PK007]
Mass balance (urine, feces)	Human	PO	MRL [redacted][c], [redacted][d]	PK014	[資料 4.2.2.5: PK014]
Excretion into milkc	Rat	PO	MRL	TT[redacted]7130	[資料 4.2.2.3: TT137130]
Drug Drug Interaction					
Human CYP450 Phenotyping	Human Liver Microsomes	In vitro	MRL	PK002	[資料 4.2.2.4: PK002]
Inhibition of Cytochromes P450	Human Liver Microsomes	In vitro	[redacted][e]	PK002	[資料 4.2.2.4: PK002]
Time-Dependent Inhibition of CYP3A4 activity	Human Liver Microsomes	In vitro	MRL	PK002	[資料 4.2.2.4: PK002]

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2.6.5.1　薬物動態試験：一覧表（続き）

Test Article: MK-3102

Type of Study	Test System	Method of Administration	Testing Facility [a]	Study Number	Location Volume
CYP3A4 and CYP1A2 induction in human hepatocytes	Human Hepatocytes	In vitro	MRL	PK002	[資料 4.2.2.4: PK002]
CYP2B6 induction in human hepatocytes	Human Hepatocytes	In vitro	[redacted] [f]	PK010	[資料 4.2.2.6: PK010]
Inhibition of Human UGT1A1 and SULT1E1	Human Liver Microsomes, Human Liver Cytosol	In vitro	MRL	PK008	[資料 4.2.2.6: PK008]
Effect on P-gp mediated transport	LLC-MDR1 cells	In vitro	[redacted] [f]	PK004	[資料 4.2.2.6: PK004]
Effect on BCRP, OATP1B1, and OATP1B3 mediated transport	Sf9 cells, MDCKII cells	In vitro	MRL	PK012	[資料 4.2.2.6: PK012]
Effect on MATE1 mediated transport	CHO-K1 cells	In vitro	MRL	PK015	[資料 4.2.2.6: PK015]
Evaluation of MK-3102 as a substrate and potential inhibitor of hOAT1, hOAT3, hOCT1 and hOCT2 mediated transport	MDCKII cells, CHO-K1 cells	In vitro	MRL	PK005	[資料 4.2.2.6: PK005]

[a] – MRL = Merck Research Laboratories.
[b] – [redacted] = [redacted]
[c] – [redacted] = [redacted]
[d] – [redacted] = [redacted]
[e] – [redacted] = [redacted]
[f] – [redacted] = [redacted]

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2.6.5.2　生体試料中濃度測定方法及びバリデーション報告書

The analytical methods and associated validation reports are described in the Pharmacokinetics Written Summary 2.6.4.

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2.6.5.3　ラット及びイヌに単回経口投与した際の薬物動態及び経口バイオアベイラビリティ

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.2: PK001]

Species:	Rat	Rat	Dog	Dog
Gender / Number of animals:	M/2	M/3	M/2	M/2
Feeding condition:	Fasted	Fasted	Fasted	Fasted
Vehicle/Formulation:	Ethanol:Water (5:95)	Ethanol:Water (5:95)	Saline	Saline
Method of administration:	IV	PO	IV	PO
Dose (mg/kg):	1	2	1	2
Sample:	Plasma	Plasma	Plasma	Plasma
Analytes:	MK-3102	MK-3102	MK-3102	MK-3102
Assay:	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
PK parameters (Mean ± SD):				
Total CL_p (mL/min/kg)	1.1	NA	0.9	NA
Vd_{ss} (L/kg)	0.77	NA	1.3	NA
$T_{1/2}$ (hr)	11 (MRT)[a]	NA	22	NA
$AUC_{0-\infty}$ (μM•hr)	38.3[a]	95.5 ± 18.5[a]	47.1	108
C_{max} (μM)	NA	8.96 ± 1.11	NA	5.94
T_{max} (hr)	NA	0.5-2.0	NA	0.5-2.0
Bioavailability, %	NA	~100	NA	~100

Values are shown as mean ± SD, or average for n=2.
NA: Not Applicable.
MRT: Mean Residence Time.
[a] Due to a secondary peak in the time vs concentration profile observed at a later time point, AUC_{0-x} and MRT were calculated instead.

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2.6.5.3 ラット及びイヌに単回経口投与した際の薬物動態及び経口バイオアベイラビリティ（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.2: PK001]

Additional Information: In rats following IV administration of MK-3102 at 1 mg/kg, the plasma clearance, steady-state volume of distribution and MRT were 1.1 mL/min/kg, 0.77 L/kg and 11 hr, respectively. Following oral administration (2 mg/kg), a mean C_{max} of 8.96 µM was observed at 0.5-2.0 hr postdose, and the bioavailability was ~100%. In dogs, the plasma clearance, steady-state volume of distribution and terminal half-life were 0.9 mL/min/kg, 1.3 L/kg and 22 hr, respectively. After a 2 mg/kg oral dose, MK-3102 was absorbed rapidly, with an average C_{max} of 5.94 µM observed at 0.5-2 hr postdose. The bioavailability was ~100%.

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2.6.5.4　組織分布

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Species:	Long-Evans (LE) Rats
Gender/Number of Animals:	M/1 per time point
Feeding condition:	Fasted
Vehicle/Formulation:	0.5% methylcellulose
Method of Administration:	P.O.
Dose (mg/kg):	2
Radionuclide:	^{14}C
Specific Activity (μCi/mg):	101
Analyte:	Radioactivity
Assay:	QWBA (Quantitative Whole Body Autoradiography), LSC
Sampling Times:	1, 4, 8, 24, 72, 168, 336, and 672 hr

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 1　Concentrations of Radioactivity in Blood and Tissues as Determined by QWBA at Specified Times After a Single Oral Administration of [^{14}C]MK-3102 to Male Long-Evans Rats (2 mg/kg)

	ng Equivalents [^{14}C]MK-3102/g							
	Time Point							
Matrix	1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr
Adrenal gland(s)	4950	2640	2170	662	159	75.2	24.0	14.1
Arterial wall	3350	1230	1050	275	82.6	30.1	ND	ND
Bile	6070	3230	ND	ND	ND	ND	ND	ND
Blood	1260	558	371	62.7	12.4	BLQ	BLQ	ND
Bone	129	78.3	72.8	BLQ	ND	ND	ND	ND
Bone marrow	3160	1360	1220	408	127	68.4	21.1	10.2
Brain cerebellum	784	316	246	50.5	10.4	BLQ	ND	ND
Brain cerebrum	862	345	285	51.8	9.28	BLQ	ND	ND
Brain medulla	833	303	264	43.4	BLQ	BLQ	ND	ND
Brain olfactory lobe	699	251	221	69.6	12.1	BLQ	ND	ND
Buccal gland(s)	4060	1590	978	173	20.9	ND	ND	ND
Bulbo-urethral gland	2180	1120	823	177	42.7	ND	ND	ND
Cecum	1890	828	1610	251	44.2	25.7	ND	ND
Contents, cecum	1320	2380	15400	690	67.5	15.5	BLQ	ND
Contents, esophageal	5590	2090	1500	388	39.0	BLQ	ND	ND
Contents, large intestine	934	NR	25500	1570	117	20.5	BLQ	ND
Contents, small intestine	37300	56400[b]	2560	454	80.3	14.9	BLQ	BLQ
Contents, stomach	30900	9120	1900	167	16.2	BLQ	BLQ	BLQ
Diaphragm	1640	837	562	89.1	31.3	BLQ	ND	ND
Epididymis	1780	904	606	284	54.5	44.7	15.4	BLQ
Esophagus	1800	754	531	83.7	34.3	9.62	ND	ND
Exorbital lacrimal gland	2900	1430	1110	351	93.4	59.1	18.6	16.6

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 1 (Cont). Concentrations of Radioactivity in Blood and Tissues as Determined by QWBA at Specified Times after a Single Oral Administration of [^{14}C]MK-3102 to Male Long-Evans Rats (2 mg/kg)

	ng Equivalents [^{14}C]-MK-3102/g							
	Time Point							
Matrix	1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr
Eye lens	74.8	364	669	2000	325	258	65.7	11.7
Eye uveal tract	35500	43800	72200	112000[a]	53200	41100	31400	24500
Eye vitreous humor	1250	2090	2530	3370	1040	1250	260	192
Eye(s)	6290	9680	14300	21500	11500	11100	5720	4440
Fascia	1630	669	455	102	36.0	23.8	10.4	ND
Fat (abdominal)	125	154	81.1	53.1	11.6	ND	ND	ND
Fat (brown)	2990	1050	425	100	27.9	13.5	13.7	13.1
Harderian gland	18000	15600	26800	2750	649	79.6	26.0	ND
Intra-orbital lacrimal gland	2900	1460	909	406	102	78.7	20.6	17.6
Kidney cortex	6710	3460	3270	2570	1520	1030	292	97.0
Kidney medulla	7420	3770	3330	2800	1930	1600	406	132
Kidney(s)	7030	3590	3240	2760	1690	1280	351	112
Large intestine	2330	1490	907	255	50.1	16.2	ND	ND
Liver	6120	3140	1890	616	256	90.0	30.5	10.4
Lung(s)	4130	1710	1410	397	262	97.8	31.7	11.8
Lymph node(s)	2340	1370	896	305	80.6	39.3	24.5	ND
Meninges	3720	1400	2760	2020	490	1220	620	584
Muscle	1110	474	377	60.7	11.9	ND	ND	ND
Myocardium	1900	878	574	149	43.6	13.1	ND	ND
Nasal turbinates	1980	858	702	260	38.1	ND	ND	ND

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 1 (Cont). Concentrations of Radioactivity in Blood and Tissues as Determined by QWBA at Specified Times after a Single Oral Administration of [^{14}C]MK-3102 to Male Long-Evans Rats (2 mg/kg)

	ng Equivalents [^{14}C]MK-3102/g							
	Time Point							
Matrix	1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr
Pancreas	2770	1220	751	205	47.5	15.6	BLQ	ND
Pituitary gland	9680	2620	3920	757	165	57.6	11.9	10.2
Preputial gland	1530	1380	1490	535	194	19.2	BLQ	ND
Prostate gland	3330	2340	1540	264	41.3	BLQ	BLQ	ND
Salivary gland(s)	4570	2490	1320	333	85.5	43.7	14.2	12.3
Salivary gland(s), parotid	2590	1370	978	180	44.5	25.3	13.8	13.2
Seminal vesicle(s)	1730	951	652	132	19.8	BLQ	ND	ND
Skin (nonpigmented)	1060	592	367	83.9	28.5	BLQ	ND	ND
Skin (pigmented)	2810	3310	5540	6120	2600	101	23.2	21.1
Small intestine	2940	1800	999	383	77.6	32.7	21.2	BLQ
Spinal cord	826	326	266	49.5	9.73	ND	ND	ND
Spleen	3570	2050	1430	448	219	118	38.8	24.7
Stomach	5010	1380	763	208	37.3	ND	ND	ND
Testis(es)	1340	988	762	244	37.5	10.7	BLQ	ND
Thymus	2370	1190	921	285	153	67.1	18.8	10.5
Thyroid	2450	1130	344	190	47.9	40.0	23.0	16.1
Urinary bladder	2130	954	550	281	34.0	BLQ	ND	ND
Urine	46500	2780	2810	294	54.2	15.2	ND	ND

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 1 (Cont). Concentrations of Radioactivity in Blood and Tissues as Determined by QWBA at Specified Times after a Single Oral Administration of [^{14}C]MK-3102 to Male Long-Evans Rats (2 mg/kg)

BLQ Below the limit of quantitation (<9.25 ng equivalents [^{14}C]MK-3102/g).
ND Not detectable (sample shape not discernible from background or surrounding tissue).
NR Not represented (tissue not present in section).
QWBA Quantitative whole-body autoradiography.
[a] Two samples of this tissue that were used to calculate this value were above the upper limit of quantitation (>90800 ng equivalents ^{14}C-MK-3102/g).

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 2　Tissue: Plasma Concentration Ratios at Specified Times After a Single Oral Administration of [^{14}C]MK-3102 to Male Long-Evans Rats (2 mg/kg)

	Tissue:Plasma Concentration Ratio							
	Time Point							
Matrix	1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr
Adrenal gland(s)	2.35	1.71	3.98	7.08	10.8	24.3	NA	NA
Arterial wall	1.59	0.799	1.93	2.93	5.62	9.71	NA	NA
Blood	0.597	0.362	0.681	0.670	0.842	NA	NA	NA
Bone	0.0612	0.0508	0.134	NA	NA	NA	NA	NA
Bone marrow	1.50	0.883	2.24	4.36	8.64	22.1	NA	NA
Brain cerebellum	0.371	0.205	0.452	0.539	0.707	NA	NA	NA
Brain cerebrum	0.408	0.224	0.524	0.554	0.631	NA	NA	NA
Brain medulla	0.395	0.197	0.485	0.463	NA	NA	NA	NA
Brain olfactory lobe	0.331	0.163	0.406	0.743	0.820	NA	NA	NA
Buccal gland(s)	1.92	1.03	1.79	1.85	1.42	NA	NA	NA
Bulbo-urethral gland	1.03	0.727	1.51	1.89	2.90	NA	NA	NA
Cecum	0.896	0.538	2.95	2.69	3.01	8.29	NA	NA
Diaphragm	0.777	0.543	1.03	0.952	2.13	NA	NA	NA
Epididymis	0.844	0.587	1.11	3.03	3.71	14.4	NA	NA
Esophagus	0.853	0.489	0.974	0.895	2.33	3.10	NA	NA
Exorbital lacrimal gland	1.37	0.929	2.04	3.75	6.36	19.1	NA	NA
Eye lens	0.0354	0.236	1.23	21.4	22.1	83.1	NA	NA
Eye uveal tract	16.8	28.4	132	1200	3620	13300	NA	NA
Eye vitreous humor	0.592	1.36	4.64	36.0	70.7	403	NA	NA
Eye(s)	2.98	6.29	26.2	230	782	3580	NA	NA

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 2 (Cont.)　Tissue: Plasma Concentration Ratios at Specified Times After a Single Oral Administration of [^{14}C]MK-3102 to Male Long-Evans Rats (2 mg/kg)

	Tissue:Plasma Concentration Ratio							
	Time Point							
Matrix	1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr
Fascia	0.773	0.435	0.835	1.09	2.45	7.68	NA	NA
Fat (abdominal)	0.0592	0.0998	0.149	0.568	0.786	NA	NA	NA
Fat (brown)	1.42	0.682	0.781	1.07	1.90	4.35	NA	NA
Harderian gland	8.53	10.1	49.2	29.4	44.1	25.7	NA	NA
Intra-orbital lacrimal gland	1.37	0.948	1.67	4.34	6.91	25.4	NA	NA
Kidney cortex	3.18	2.25	6.00	27.5	103	332	NA	NA
Kidney medulla	3.52	2.45	6.11	29.9	131	516	NA	NA
Kidney(s)	3.33	2.33	5.94	29.5	115	413	NA	NA
Large intestine	1.10	0.968	1.66	2.73	3.41	5.23	NA	NA
Liver	2.90	2.04	3.47	6.58	17.4	29.0	NA	NA
Lung(s)	1.96	1.11	2.59	4.25	17.9	31.5	NA	NA
Lymph node(s)	1.11	0.890	1.64	3.26	5.48	12.7	NA	NA
Meninges	1.76	0.909	5.06	21.6	33.4	394	NA	NA
Muscle	0.526	0.308	0.692	0.649	0.809	NA	NA	NA
Myocardium	0.900	0.570	1.05	1.59	2.96	4.23	NA	NA
Nasal turbinates	0.938	0.557	1.29	2.78	2.59	NA	NA	NA
Pancreas	1.31	0.792	1.38	2.19	3.23	5.03	NA	NA
Pituitary gland	4.59	1.70	7.19	8.08	11.2	18.6	NA	NA
Preputial gland	0.725	0.896	2.73	5.72	13.2	6.19	NA	NA
Prostate gland	1.58	1.52	2.83	2.82	2.81	NA	NA	NA

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 2 (Cont.)　Tissue: Plasma Concentration Ratios at Specified Times After a Single Oral Administration of [^{14}C]MK-3102 to Male Long-Evans Rats (2 mg/kg)

	Tissue:Plasma Concentration Ratio							
	Time Point							
Matrix	1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr
Salivary gland(s)	2.17	1.62	2.42	3.55	5.82	14.1	NA	NA
Salivary gland(s), parotid	1.23	0.890	1.80	1.92	3.03	8.16	NA	NA
Seminal vesicle(s)	0.820	0.618	1.20	1.41	1.34	NA	NA	NA
Skin (nonpigmented)	0.502	0.384	0.674	0.896	1.94	NA	NA	NA
Skin (pigmented)	1.33	2.15	10.2	65.4	177	32.5	NA	NA
Small intestine	1.39	1.17	1.83	4.09	5.28	10.5	NA	NA
Spinal cord	0.391	0.212	0.489	0.529	0.662	NA	NA	NA
Spleen	1.69	1.33	2.62	4.79	14.9	38.0	NA	NA
Stomach	2.37	0.896	1.40	2.22	2.54	NA	NA	NA
Testis(es)	0.635	0.642	1.40	2.60	2.55	3.46	NA	NA
Thymus	1.12	0.773	1.69	3.04	10.4	21.7	NA	NA
Thyroid	1.16	0.734	0.631	2.03	3.26	12.9	NA	NA
Urinary bladder	1.01	0.620	1.01	3.00	2.31	NA	NA	NA
NA　Not applicable.								

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 3　Percent of Radioactive Dose in Blood and Tissues Determined by QWBA at Specified Times After a Single Oral Dose of [^{14}C]MK-3102 to Male Long-Evans Rats (2 mg/kg)

	Percent of Dose Administered							
	Time Point							
Matrix	1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr
Adrenal gland(s)	0.0431	0.0226	0.0191	0.00554	0.00138	0.000669	0.000204	0.000120
Arterial wall	NA	NA	NA	NA	NA	NA	NA	NA
Bile	NA	NA	NA	NA	NA	NA	NA	NA
Blood	4.29	1.87	1.28	0.205	0.0420	NA	NA	NA
Bone	0.314	0.187	0.179	NA	NA	NA	NA	NA
Bone marrow	0.538	0.228	0.210	0.0667	0.0215	0.0119	0.00351	0.00169
Brain cerebellum	0.0427	0.0170	0.0135	0.00264	0.000564	NA	NA	NA
Brain cerebrum	0.169	0.0666	0.0565	0.00975	0.00181	NA	NA	NA
Brain medulla	0.0364	0.0130	0.0117	0.00182	NA	NA	NA	NA
Brain olfactory lobe	0.00745	0.00263	0.00238	0.000712	0.000128	NA	NA	NA
Buccal gland(s)	NA	NA	NA	NA	NA	NA	NA	NA
Bulbo-urethral gland(s)	NA	NA	NA	NA	NA	NA	NA	NA
Cecum	0.335	0.144	0.288	0.0427	0.00780	0.00465	NA	NA
Diaphragm	NA	NA	NA	NA	NA	NA	NA	NA
Epididymis	0.135	0.0675	0.0464	0.0207	0.00412	0.00347	0.00114	NA
Esophagus	NA	NA	NA	NA	NA	NA	NA	NA
Exorbital lacrimal gland	NA	NA	NA	NA	NA	NA	NA	NA
Eye lens	0.000436	0.00209	0.00394	0.0112	0.00189	0.00154	0.000375	0.0000665
Eye uveal tract	1.62	1.96	3.32	4.90	2.41	1.91	1.40	1.09
Eye vitreous humor	NA	NA	NA	NA	NA	NA	NA	NA

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 3 (Cont.)　Percent of Radioactive Dose in Blood and Tissues Determined by QWBA at Specified Times After a Single Oral Dose of [^{14}C]MK-3102 to Male Long-Evans Rats (2 mg/kg)

	Percent of Dose Administered							
	Time Point							
Matrix	1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr
Eye(s)	0.286	0.434	0.657	0.940	0.522	0.516	0.254	0.197
Fascia	NA	NA	NA	NA	NA	NA	NA	NA
Fat (abdominal)	0.438	0.531	0.287	0.179	0.0404	NA	NA	NA
Fat (brown)	0.189	0.0653	0.0272	0.00608	0.00176	0.000871	0.000845	0.000807
Harderian gland	0.799	0.682	1.20	0.117	0.0287	0.00361	0.00113	NA
Intra-orbital lacrimal gland	NA	NA	NA	NA	NA	NA	NA	NA
Kidney(s)	2.90	1.46	1.35	1.09	0.694	0.540	0.141	0.0451
Large intestine	0.783	0.493	0.308	0.0824	0.0168	0.00557	NA	NA
Liver	15.0	7.58	4.68	1.45	0.624	0.226	0.0731	0.0248
Lung(s)	0.964	0.393	0.332	0.0891	0.0610	0.0233	0.00722	0.00269
Lymph node(s)	NA	NA	NA	NA	NA	NA	NA	NA
Meninges	NA	NA	NA	NA	NA	NA	NA	NA
Muscle	24.6	10.3	8.42	1.29	0.262	NA	NA	NA
Myocardium	0.298	0.136	0.0911	0.0224	0.00682	0.00211	NA	NA
Nasal turbinates	NA	NA	NA	NA	NA	NA	NA	NA
Pancreas	0.473	0.205	0.129	0.0336	0.00807	0.00272	NA	NA
Pituitary gland	0.0135	0.00359	0.00551	0.00101	0.000229	0.0000819	0.0000162	0.0000138
Preputial gland	0.0352	0.0312	0.0346	0.0118	0.00444	0.000451	NA	NA
Prostate gland	0.131	0.0904	0.0610	0.00995	0.00162	NA	NA	NA
Salivary gland(s)	0.465	0.249	0.135	0.0325	0.00866	0.00453	0.00141	0.00122

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 3 (Cont.)　Percent of Radioactive Dose in Blood and Tissues Determined by QWBA at Specified Times After a Single Oral Dose of [^{14}C]MK-3102 to Male Long-Evans Rats (2 mg/kg)

	Percent of Dose Administered							
	Time Point							
Matrix	1 hr	4 hr	8 hr	24 hr	72 hr	168 hr	336 hr	672 hr
Salivary gland(s), parotid	NA	NA	NA	NA	NA	NA	NA	NA
Seminal vesicle(s)	0.133	0.0720	0.0506	0.00977	0.00151	NA	NA	NA
Skin (nonpigmented)	6.08	3.34	2.13	0.462	0.163	NA	NA	NA
Skin (pigmented)	16.1	18.7	32.1	33.7	14.9	0.590	0.130	0.118
Small intestine	3.15	1.90	1.08	0.393	0.0827	0.0357	0.0221	NA
Spinal cord	0.0803	0.0312	0.0262	0.00462	0.000943	NA	NA	NA
Spleen	0.457	0.258	0.185	0.0551	0.0279	0.0154	0.00485	0.00308
Stomach	1.25	0.340	0.193	0.0500	0.00930	NA	NA	NA
Testis(es)	0.671	0.487	0.385	0.117	0.0187	0.00549	NA	NA
Thymus	0.225	0.111	0.0882	0.0259	0.0145	0.00651	0.00174	0.000975
Thyroid	0.00672	0.00305	0.000952	0.000500	0.000131	0.000112	0.0000616	0.0000430
Urinary bladder	0.0321	0.0142	0.00838	0.00406	0.000511	NA	NA	NA
Total	83.1	52.5	59.4	45.5	20.0	3.91	2.04	1.48

NA　Not applicable.
QWBA　Quantitative whole-body autoradiography.
Note: Tissue weights used to determine the percent of radioactive dose were derived from values cited in the literature or from data obtained at Covance.

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 4　Estimated Pharmacokinetic Parameters for Selected Tissues in Male Long-Evans Rats Following a Single Oral Dose of [^{14}C]MK-3102 (2 mg/kg)

Matrix	AUC_{0-t} (ng eq·hr/g)	$AUC_{0-\infty}$ (ng eq·hr/g)	C_{max} (ng eq/g)	T_{max} (hr)	$t_{1/2}$ (hr)
Adrenal gland(s)	91815	95446	4950	1.00	178
Arterial wall	37697	39749	3350	1.00	47.2
Bile	16985	32345	6070	1.00	3.30
Blood	10487	10742	1260	1.00	14.3
Blood (LSC)	11580	11714	1210	1.00	86.3
Bone	678	1607	129	1.00	8.85
Bone marrow	61540	64007	3160	1.00	168
Brain cerebellum	7000	7212	784	1.00	14.1
Brain cerebrum	7662	7839	862	1.00	13.2
Brain medulla	5714	6141	833	1.00	6.82
Brain olfactory lobe	7004	7276	699	1.00	15.6
Buccal gland(s)	29503	29844	4060	1.00	11.3
Bulbo urethral gland	23199	24119	2180	1.00	14.9
Cecum	35226	37014	1890	1.00	48.2
Diaphragm	15432	16868	1640	1.00	31.8
Epididymis	32990	36093	1780	1.00	140
Esophagus	17159	17810	1800	1.00	46.9
Exorbital lacrimal gland	55139	60959	2900	1.00	243
Eye(s)	5358020	7971243	21500	24.0	408
Eye lens	148092	150121	2000	24.0	120
Eye uveal tract	25814700	50679695	112000[a]	24.0	703

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 4 (Cont.)　Estimated Pharmacokinetic Parameters for Selected Tissues in Male Long-Evans Rats Following a Single Oral Dose of [^{14}C]MK-3102 (2 mg/kg)

	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}	T_{max}	$t_{1/2}$
Matrix	(ng eq·hr/g)	(ng eq·hr/g)	(ng eq/g)	(hr)	(hr)
Eye vitreous humor	480611	693391	3370	24.0	768
Fascia	20023	22222	1630	1.00	147
Fat (abdominal)	3578	3956	154	4.00	22.6
Fat (brown)	26549	NC	2990	1.00	NC
Harderian gland	506019	507827	26800	8.00	48.2
Intra-orbital lacrimal gland	58872	64664	2900	1.00	228
Kidney(s)	545253	566776	7030	1.00	133
Kidney cortex	475750	495195	6710	1.00	139
Kidney medulla	625583	651902	7420	1.00	138
Large intestine	31490	32386	2330	1.00	38.3
Liver	101587	104107	6120	1.00	168
Lungs	82793	85731	4130	1.00	173
Lymph node(s)	41244	46916	2340	1.00	160
Meninges	555252	NC	3720	1.00	NC
Muscle	9877	10101	1110	1.00	13.1
Myocardium	21149	21955	1900	1.00	42.6
Nasal turbinates	23217	24057	1980	1.00	15.3
Pancreas	28049	28967	2770	1.00	40.8
Pituitary gland	116150	NC	9680	1.00	NC
Plasma (LSC)	19262	19400	2110	1.00	30.7
Preputial gland	54800	55625	1530	1.00	29.8

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Table 4 (Cont.)　Estimated Pharmacokinetic Parameters for Selected Tissues in Male Long-Evans Rats Following a Single Oral Dose of [^{14}C]MK-3102 (2 mg/kg)

Matrix	AUC_{0-t} (ng eq·hr/g)	$AUC_{0-\infty}$ (ng eq·hr/g)	C_{max} (ng eq/g)	T_{max} (hr)	$t_{1/2}$ (hr)
Prostate gland	39689	40412	3330	1.00	12.1
Salivary gland(s)	59280	63260	4570	1.00	224
Salivary gland(s), parotid	37754	44916	2590	1.00	376
Seminal vesicle(s)	18008	18367	1730	1.00	12.6
Skin (nonpigmented)	11231	11916	1060	1.00	16.7
Skin (pigmented)	478368	NC	6120	24.0	NC
Small intestine	46110	50668	2940	1.00	149
Spinal cord	7271	7462	826	1.00	13.6
Spleen	88222	94055	3570	1.00	164
Stomach	30031	30869	5010	1.00	15.6
Testis(es)	24780	25175	1340	1.00	25.6
Thymus	53610	55729	2370	1.00	140
Thyroid	35605	44343	2450	1.00	376
Urinary bladder	22907	23687	2130	1.00	15.9
Urine	144870	145649	46500	1.00	35.5

NC　Not calculated.
[a]　It should be noted that C_{max} for the uveal tract may have been underestimated since two samples used to calculate this value were above the upper limit of quantitation.

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2.6.5.4 組織分布（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK006]

Additional Information: Following a single 2-mg/kg oral dose of [^{14}C]MK-3102 to pigmented male Long-Evans rats, C_{max} values were observed at 1 hr postdose for blood (LSC analysis), plasma, and most tissues, indicating rapid absorption and distribution of drug-derived radioactivity into tissues. Concentrations of radioactivity were sustainable in most tissues for at least 168 hr postdose with measurable amounts present in 35 tissues at this sampling time. Although measurable amounts of radioactivity were still present in 22 tissues including the uveal tract, lens of the eye, meninges, and pigmented skin tract at 672 hr postdose, the radioactivity in tissues only represented ~1.5% of the dose administered. The presence of radioactivity in bile and urine illustrated that biliary and renal excretion were routes of elimination for [^{14}C]MK-3102-derived radioactivity in the rat.

Excluding GI tract contents, bile, and urine, tissues with the highest C_{max} concentrations of radioactivity were uveal tract, Harderian gland, eye, pituitary gland, and kidney medulla.

Concentrations of [^{14}C]MK-3102-related radioactivity in the uveal tract, pigmented skin, and meninges reached maximum levels at 24 hr postdose and then slowly declined to the lowest observed levels by the last sampling time of 672 hr postdose. The data illustrated that [^{14}C]MK-3102-related radioactivity exhibited an affinity for pigmented tissues containing melanin and that this association was reversible.

Radioactivity was measurable in non-circumventricular CNS tissues through 72 hr postdose and testis through 168 hours postdose. The data illustrated that [^{14}C]MK-3102-derived radioactivity distributed into these tissues having blood barriers.

[^{14}C]MK-3102-related radioactivity also appeared to be sustained in tissues with high DPP-4 activity, including kidney, liver, salivary glands, spleen, lungs etc., with an elimination $T_{1/2}$ around 140 – 220 hr, compared to 86 hr in blood.

Tissues with the highest exposures based on $AUC_{0-\infty}$ values were the uveal tract, eye, vitreous humor of the eye, kidney medulla, and kidney. Bone, abdominal fat, non-circumventricular CNS tissues, muscle, and blood had the lowest $AUC_{0-\infty}$ values. Elimination $t_{1/2}$ values ranged from 3.30 hr in bile to 768 hr in the vitreous humor of the eye. The elimination $t_{1/2}$ for blood (LSC analysis) was 86.3 hr. Elimination $t_{1/2}$ and $AUC_{0-\infty}$ values were not determined for brown fat, meninges, pigmented skin, and pituitary gland because definitive elimination phases were not apparent for these tissues.

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2.6.5.5　妊娠動物を用いた薬物動態試験

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.3: TT[redacted]7130]
[資料 4.2.2.3: TT[redacted]7140]

Placenta Transfer

Species: Rat, Rabbit
Gestation Day/Number of Animals: Day 20/8 per dose
Vehicle/Formulation: 0.5% (w/v) methylcellulose in water
Method of Administration: Oral gavage
Analyte: MK-3102
Assay: LC-MS/MS

Species - Dose (mg/kg/day):	Rat-10	Rat -100
Analyte	MK-3102	MK-3102
Gestation day	Day 20	Day 20
Concentration (μM)[a]:		
Maternal plasma		
2 hour (n=4)	25.3 ± 2.68	164 ± 9.59
24 hours (n=4)	8.00 ± 1.68	57.7 ± 6.74
Fetal plasma		
2 hour	14.2 ± 1.13	101 ± 2.22
24 hours	4.07 ± 0.665	37.3 ± 2.66
Ratio [b] (Fetal/Maternal)		
2 hour	0.571 ± 0.0397	0.618 ± 0.0267
24 hours	0.522 ± 0.0246	0.672 ± 0.0859

[a] Mean ± SEM.
[b] Mean ± SEM of the individual fetal plasma concentration divided by the corresponding maternal plasma concentration.

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2.6.5.5 妊娠動物を用いた薬物動態試験（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.3: TT[redacted]7130]
[資料 4.2.2.3: TT[redacted]7140]

Placenta Transfer (Cont.)

Species: Rat, Rabbit
Gestation Day/Number of Animals: Day 20/8 per dose
Vehicle/Formulation: 0.5% (w/v) methylcellulose in water
Method of Administration: Oral gavage
Analyte: MK-3102
Assay: LC-MS/MS

Species - Dose (mg/kg/day):	Rabbit-50
Analyte	MK-3102
Gestation day:	Day 20
Concentration (μM)[a]:	
Maternal plasma	
2 hours (n=4)	85.3 ± 2.62
24 hours (n=4)	23.2 ± 5.12
Fetal plasma	
2 hours	47.0 ± 3.62
24 hours	13.4 ± 3.07
Ratio [b] (Fetal/Maternal)	
2 hours	0.550 ± 0.0264
24 hours	0.572 ± 0.0154

[a] Mean ± SEM.
[b] Mean ± SEM of the individual fetal plasma concentration divided by the corresponding maternal plasma concentration.

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2.6.5.5　妊娠動物を用いた薬物動態試験（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.3: TT[redacted]7130]
[資料 4.2.2.3: TT[redacted]7140]

Placenta Transfer (Cont.)

Additional Information: MK-3102 was orally administered to pregnant rats from gestation days (GD) 6 through 20 at doses of 10 or 100 mg/kg/day (n=8 rats/dose), and MK-3102 was detected in the maternal and fetal plasma at 2 and 24 hr postdose on GD 20. All rats survived until scheduled study termination. The placental transfer of MK-3102 was approximately 57% and 52% at 2 hr and 24 hr, respectively, after the 10 mg/kg/day dose on GD 20. Placental transfer of MK-3102 was approximately 62% and 67% at 2 hr and 24 hr, respectively, after the 100 mg/kg/day dose on GD 20.

MK-3102 was orally administered to 8 pregnant Dutch Belted rabbits from GD 7 through 20 at a dose of 50 mg/kg/day, and MK-3102 was observed in the maternal and fetal plasma at 2 and 24 hr post-dose on GD 20. All animals survived until scheduled termination. The placental transfer of MK-3102 was approximately 55% at 2 hr postdose and approximately 57% at 24 hr postdose.

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2.6.5.6　血漿蛋白結合率

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

Study System: In Vitro
Test System and Method: Rat, Dog, Rhesus monkey, Cyno monkey, Mouse, Rabbit and Human Plasma
Radionuclide: ^{3}H
Analyte: Total radioactivity

Table 1　In Vitro Reversible Binding of [^{3}H]MK-3102 to Plasma Proteins

Concentration	Percentage (%) Unbound in Plasma (N=3)[a]						
	CD1 Mouse	Rat	Dog	Rhesus Monkey	Human	Cyno Monkey	Rabbit
1 µM	36[b]	14±0.5	41[b]	84[c]	66±2.6	85±3.6[e]	80±3.6[e]
10 µM	38±0.5	15±0.9	42±1.5	86±2.1	68±0.8	87±1.9[e]	76±0.8[d]
100 µM	38[c]	16±0.3	46±0.4	86±0.4	71±2.6	88±1.3[e]	77±15[e]
Average[f]	38	15	43	85	68	87	78

[a] Plasma protein binding in CD-1 mouse, rat, dog, rhesus monkey and human was performed using Harvard Equilibrium Dialyzer plate. Plasma protein binding in cyno monkey and Dutch-Belted rabbit was performed using HTDialysis Equilibrium Dialyzer plate. Values shown are mean ± SD from N=3 replicates.
[b] Mean value from duplicate determinations (N=2).
[c] Value from single determination (N=1)
[d] Values shown are mean ± SD from N=4 replicates.
[e] Values shown are mean ± SD from N=5 replicates.
[f] Mean value of percentage unbound from the three concentrations tested.

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2.6.5.6 血漿蛋白結合率（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

Study System: In Vitro
Test System and Method: Human Plasma
Radionuclide: ^{3}H
Analyte: Total radioactivity

Table 2　Concentration Dependent In Vitro Reversible Binding of [^{3}H]MK-3102 to Human Plasma Proteins

Concentration (nM)	Percentage (%) Unbound in Human Plasma (N=5)[a]
1	25±2.5
5	36±1.0
10	43±2.6
25	56±0.4
50	64±3.3
100	71±1.3
250	76±2.8
1000	76±2.5

[a] Plasma protein binding in human plasma was performed using HTDialysis Equilibrium Dialyzer plate. Values shown are mean ± SD from N=5 replicates.

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2.6.5.6 血漿蛋白結合率（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

Study System: In Vitro
Test System and Method: DPP-4 Knock-out Mouse plasma with or without spiked human recombinant DPP-4 Enzyme
Radionuclide: ^{3}H
Analyte: Total radioactivity

Table 3　In Vitro Reversible Binding of [^{3}H]MK-3102 to DPP-4 Knock-out (KO) Mouse Plasma Proteins With and Without Spiked Human Recombinant DPP-4 Enzyme

Concentration (nM)	Percentage (%) Unbound in DPP-4 KO Mouse Plasma (N=3)[a]	
	No Human DPP-4	With Human DPP-4[b]
1	32±0.6	23±2.1
5	38±2.1	24±1.9
10	37±2.4	26±1.2
25	38±2.6	28±0.7
50	41±0.5	34±1.0
100	41±1.2	36±0.6
250	40±0.1	41±0.8
1000	39±0.6	42±1.0

[a] Plasma protein binding was performed using 96-well RED Equilibrium Dialyzer Plate. Values shown are mean ± SD from N=3 replicates.
[b] Final concentration of human DPP-4 was 6 nM.

Additional Information: MK-3102 exhibited no concentration-dependent protein binding in the knock-out mouse plasma, but showed concentration dependency in the knock-out mouse plasma spiked with human DPP-4.

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2.6.5.6 血漿蛋白結合率（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK011]

Study System: In Vitro
Test System and Method: Human plasma from renal insufficient patients and matched healthy subjects
Radionuclide: ^{3}H
Analyte: Total radioactivity

Table 4　Reversible Protein Binding of [^{3}H]MK-3102 in Pooled Plasma from Individual Panel[a]

MK-3102 (nM)	Percent (%) Unbound (mean±SD, N=3)[b]										
	Panel A (mild)	Panel B (control)	Panel C (moderate)	Panel D (control)	Panel E (severe)	Panel F (control)	Panel G (ESRD per 1)	Panel G (ESRD per 2)	Panel H (control)	Panel E[c] (excluding sub 29)	Panel F[d] (control)
1	18±7.0	14±1.5	20±3.4	20±3.0	54±1.1	30±3.6	25[e]	24±1.5	26±1.6	23±2.4	27±4.9
5	15[e]	17±2.1	22±0.1	26±2.0	56±4.1	29±0.2	22±0.5	31±1.1	28±4.3	25±1.4	29±4.4
10	23±1.5	28±5.4	31±2.3	36±2.3	60±1.2	34±2.4	31±1.6	39±1.3	36±3.5	34±0.9	37±3.7
25	42±1.8	40±0.7	48±2.9	52±0.7	61±4.6	44±5.7	45±1.7	53±0.8	48±1.8	51±0.8	53±2.7
50	55±2.1	54±3.0	58±2.9	57±3.6	65±4.1	54±2.3	54±3.2	58±1.9	56±2.9	61±1.1	61±5.6
100	62±6.1	64±8.3	65±1.8	66±1.5	72±5.9	61±1.4	58±2.2	65±2.3	59±2.7	67±0.3	65±0.6
250	65±0.7	64±3.7	70±5.6	73±4.0	72±2.4	65±3.1	62±2.4	68±1.8	62±1.2	68±2.8	69±4.7
1000	67±1.5	68±1.2	67±5.0	71±2.7	71±1.7	65±4.2	62±2.8	68±2.1	65±3.9	71±1.9	68±3.0

[a] Panel A: subjects with mild renal impairment
Panel B: healthy subjects to match Panel A
Panel C: subjects with moderate renal impairment
Panel D: healthy subjects to match Panel C
Panel E: subjects with severe renal impairment
Panel F: healthy subjects to match Panel E
Panel G: subjects with end-stage renal disease (ESRD); per 1: MK-3102 administered after dialysis; per 2: MK-3102 administered before dialysis
Panel H: healthy subjects to match Panel G
[b] Plasma protein binding was performed using 96-well RED Equilibrium Dialyzer Plate.
[c] Panel E plasma samples were pooled from individuals within the panel excluding subject 29.
[d] Panel F was repeated side-by-side with Panel E pooled sample excluding subject 29.
[e] Mean value from N=2 determinations.

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2.6.5.6 血漿蛋白結合率（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.3: PK011]

Additional Information: The reversible plasma protein binding of [^{3}H]MK-3102 (1 nM to 1000 nM) was determined in pooled plasma samples for each panel, except that in a few cases, protein binding was also determined in individual plasma samples from selected panels as described below. The overall recovery from the experiments was greater than 75%.

In patients with mildly impaired renal function, the percent unbound increased from 18% at 1 nM to 67% at 1000 nM (Panel A). Similarly, the percent unbound increased from 14% at 1 nM to 68% at 1000 nM in the matched control healthy subjects (Panel B).

In patients with moderately impaired renal function, the percent unbound increased from 20% at 1 nM to 67% at 1000 nM (Panel C), and similar concentration dependent increase in percent unbound was observed in the matched control subjects (Panel D).

In patients with severely impaired renal function (Panel E), however, the concentration-dependent trend appeared to be less obvious, with percent unbound of 54% at 1 nM, much greater than seen in Panel F (matched control subjects) as well as other panels. Additional studies were conducted to determine the unbound fraction in plasma from individual subject in Panel E, in order to rule out the possibility of any outlier. The result indicated that subject 29 in Panel E is markedly different from all the other subjects, with percent unbound nearly unchanged across the concentration range tested (71% at 1 nM to 72% at 1000 nM). Exclusion of this outlier resulted in a comparable concentration unbound fraction relationship for the panel as observed in Panel F. In a separate study, subject 29 was shown to have a much lower plasma DPP-4 activity, compared to other subjects.

In patients with end stage renal disease (ESRD), pre-dose samples were collected either post-dialysis (Period 1) or pre-dialysis (Period 2). Protein binding in pooled plasma samples from Panel G (both Periods) indicated similar increase in percent unbound with increasing concentrations, as seen in the matched control subjects (Panel H). Studies were also conducted with individual plasma sample from Panel G Period 2, and all the subjects showed similar protein binding curves, further supporting that the outlier observed in Panel E (subject 29) does not appear to be related to renal functions.

In summary, the reversible plasma protein binding of MK-3102 is comparable between patients with varying degree of renal functions and their matched control subjects. Thus, the plasma protein binding of MK-3102 is not altered by renal impairment.

C Confidential

2.6.5.6 血漿蛋白結合率（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: PK013]

Study System: In Vitro
Test System and Method: Human plasma from hepatic insufficient and healthy subjects
Radionuclide: ^{3}H
Analyte: Total radioactivity

Table 5　Reversible Protein Binding of [^{3}H]MK-3102 in Pooled Plasma from Panel A & B[a]

	Unbound Fraction (mean±SD, N=3)[b]	
Concentration (nM)	Panel A	Panel B
1	0.19±0.01	0.20±0.01
5	0.25±0.02	0.28±0.01
10	0.33±0.02	0.39±0.01
25	0.54±0.00	0.58±0.01
50	0.70±0.01	0.67±0.01
100	0.74±0.02	0.72±0.02
250	0.80±0.03	0.75±0.01
1000	0.80±0.02	0.83±0.01

[a] Panel A: Moderate hepatic impaired patients.
Panel B: Healthy control subjects.
[b] Plasma protein binding was determined in vitro using equilibrium dialysis method.

Additional Information: The reversible plasma protein binding of [^{3}H]MK-3102 (1 nM to 1000 nM) was determined in pooled plasma samples for each panel. The overall recovery from the experiments was greater than 80%.

The reversible plasma protein binding of MK-3102 is comparable between patients with impaired hepatic function and their matched control subjects. Thus, the plasma protein binding of MK-3102 is not altered by hepatic impairment.

C Confidential

2.6.5.7　血球移行率

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

Study System: In Vitro
Test System and Method: Blood and Plasma from rat, dog, monkey, and human
Radionuclide: ^{3}H
Analyte: Total radioactivity

Table 1　In Vitro Blood-to-Plasma Concentration Ratio of [^{3}H]MK-3102[a]

Concentration	Blood-to-Plasma Concentration Ratio			
	Rat	Dog	Monkey	Human
1 μM	0.7	0.9	1.2	0.9
10 μM	0.6	0.9	1.2	0.9
Average	0.6	0.9	1.2	0.9

[a] LSC-Liquid scintillation counting.
The blood to plasma concentration ratios are obtained using an average of n=3 measurements of blood concentration and an average of n=3 measurements of plasma concentration.

Additional Information: The blood to plasma concentration ratios for [^{3}H]MK-3102 (1 and 10 μM) in rat, dog, monkey, and human blood were 0.6, 0.9, 1.2, and 0.9, respectively.

C Confidential

2.6.5.8　P-糖蛋白を介した輸送及び in vitro 受動的膜透過性

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

Study System: In Vitro
Test System and Method: LLC-PK1 cell lines expressing the mouse (LLC-Mdr1a) and human (LLC-MDR1) P-glycoprotein, Liquid scintillation counting ([^{3}H]MK-3102), t=3 hr

Table 1　Transport of MK-3102 in LLC-PK1 Cells (3 hr Incubation)

Compound	Concentration (μM)	P_{app} (B-A)/P_{app} (A-B) Ratio (N=1)			P_{app} x 10^{-6} (cm/s) (N=1) LLC-PK1
		LLC-PK1[a]	LLC-MDR1	LLC-Mdr1a	
MK-3102	0.1	0.9	1.2	2.7	24
MK-3102	0.5	1.0	1.1	2.4	27
MK-3102	1	1.0	1.0	2.4	27
Verapamil	5	1.3	4.1	7.1	31

[a] Control cells.

Additional Information: MK-3102 exhibited high membrane permeability in control LLC-PK1 cells, with a P_{app} of ~26 x 10^{-6} cm/s. At concentrations ranging from 0.1 to 1 μM, the B-A/A-B ratio of MK-3102 in parental LLC-PK1 was 0.9-1.0, suggesting little or no vectorial transport of MK-3102 was observed. The B-A/A-B ratio in LLC-MDR1 expressing cells was 1.0-1.2, indicating that MK-3102 is not a substrate for human MDR1 P-gp. The B-A/A-B ratio in LLC-Mdr1a expressing cells was 2.4-2.7, suggesting that MK-3102 is a weak substrate of mouse Mdr1a P-gp. The B-A/A-B ratios measured in the LLC-PK1, LLC-MDR1 and LLC-Mdr1a cells for the positive control verapamil were 1.3, 4.1, and 7.1, respectively, confirming the functionality of the cell lines.

C Confidential

2.6.5.9　In vivo 代謝

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK009]
[資料 4.2.2.5: PK003]
[資料 4.2.2.5: PK007]
[資料 4.2.2.5: PK014]

Table 1

Species:	Rat	Rat	Rat	Dog	Human
Gender/Number of Animals:	M/4	M/3	M/2	M/3	M/6
Feeding Condition:	Fasted	Fasted	Fasted	Fasted	Fasted
Vehicle/Formulation:	Saline	0.5% methylcellulose	Saline	0.5% methylcellulose	Aqueous solution
Method of Administration:	PO	PO	PO	PO	PO
Dose:	2 mg/kg	20 mg/kg	2 mg/kg	2 mg/kg	25 mg
Radionuclide:	^{3}H	^{3}H	^{3}H	^{14}C	^{14}C
Specific activity, µCi/mg:	198	7.8	188	7	0.084
Biological samples:	Urine, Bile, Feces	Plasma	Urine	Bile, Urine, Feces, plasma	Urine, Feces, Plasma
Analytes:	MK-3102 and metabolites				
Assays:	LC-MS/MS with radiometric detection, Accelerator Mass Spectrometer (AMS)				

C Confidential

2.6.5.9 In vivo 代謝（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK009]
[資料 4.2.2.5: PK003]
[資料 4.2.2.5: PK007]
[資料 4.2.2.5: PK014]

Table 2　The Presence of MK-3102 and its Metabolites in Plasma and/or Excreta in Nonclinical Species and in Humans Following P.O. Administration of MK-3102

Species	Rat				Dog				Human		
Biological matrix	Plasma (Pl)	Bile (B)	Urine (U)	Feces (F)	Pl	B	U	F	Pl	U	F
MK-3102	X	X	X	N.A.	X	X	X	N.A.	X	X	N.A.
M1	X	-	X	N.A.	-	-	-	N.A.	-	X	N.A.
M2	X	X	X	N.A.	-	-	-	N.A.	-	-	N.A.
M3	X	X	X	N.A.	-	-	-	N.A.	-	X	N.A.
M4	-	-	-	N.A.	X	-	-	N.A.	-	-	N.A.
M5	-	-	X	N.A.	-	-	-	N.A.	-	X	N.A.
M6	-	-	X	N.A.	-	-	-	N.A.	-	X	N.A.

X: Detected by LC/MS and radiometric detection.
-: Not Detected.
N.A.: Not Analyzed.

C Confidential

2.6.5.9 In vivo 代謝（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.4: PK009]
[資料 4.2.2.5: PK003]
[資料 4.2.2.5: PK007]
[資料 4.2.2.5: PK014]

Additional Information: Following oral administration of [^{3}H]MK-3102 (2 mg/kg) to male bile-duct cannulated rats, MK-3102 was cleared primarily via renal excretion of unchanged parent (~61% of the recovered dose), with metabolism as a minor clearance route (~28% of the recovered dose). In rat urine, unchanged parent was the major radioactive component, representing 86% of the urinary radioactivity. Metabolites detected in urine were all oxidized derivatives, including an oxidative metabolite with addition of 18 Da at the pyrrolopyrazole tetrahydropyran moiety (M1), a ring opened carboxylic acid derivative (M2), a di-hydroxylated derivative (M3), a mono-hydroxylated derivative (M5), and an oxidative metabolite with addition of 18 Da at the pyrrolopyrazole moiety (M6). In rat bile, M2 and M3 were the major radioactive components (~16% and 56% of the total radioactivity, respectively), with unchanged parent as a minor component (19% of the total radioactivity). One radioactive peak (RT: 15.5 min) was not identified, representing <2% of the recovered dose. Thus, in rats, the primary route of metabolism of MK-3102 was *via* oxidation. Following an oral dose of [^{3}H]MK-3102 at 20 mg/kg to intact rats, parent compound was the major circulating component in rat plasma at 2 hr, 6hr, and 24 hr post dose (>99% of the total circulating radioactivity). Minor metabolites detected in rat plasma included M1, M2, and M3 (<1% of the total circulating radioactivity).

Following oral administration of [^{14}C]MK-3102 at 2 mg/kg to male bile-duct cannulated dogs, MK-3102 was primarily cleared via renal excretion of unchanged parent (~82% of the recovered dose). In dog urine, unchanged parent was the only radioactive component detected, representing 100% of the urinary radioactivity. No metabolites were detected in dog urine. In dog bile, unchanged parent represented ~92% of the total radioactivity, accounting for ~6.4% of the recovered dose. One peak (RT: 21.6 min) was not identified, representing <1% of the recovered dose. In dog plasma, unchanged was the major circulating component at 2 hr, 6hr, and 24 hr post dose (>87% of the total circulating radioactivity). A minor sulfonamide hydrolysis product (M4) was also detected in dog plasma (<13% of the total circulating radioactivity).

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2.6.5.9 In vivo 代謝（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.4: PK009]
[資料 4.2.2.5: PK003]
[資料 4.2.2.5: PK007]
[資料 4.2.2.5: PK014]

Additional Information (Cont.): The identity of M4 metabolite was confirmed based on the retention time and LC-MS/MS data for the M4 metabolite and a reference standard [redacted].

The metabolism of MK-3102 was evaluated in six healthy male subjects following administration of single 25 mg oral solution dose of [^{14}C]MK-3102 (~2.5 µCi). Similar to that observed in preclinical species, in humans, MK-3102 was eliminated primarily via renal excretion of unchanged parent, with a small contribution of metabolism. Urine contained mostly the unchanged parent, ~89% of the urinary radioactivity. The remainder of the radioactivity in urine was comprised of several oxidative metabolites, including M1, M3, M5 and M6, each representing less than 3% of the radioactivity. These metabolites were also detected in the rat. In human plasma, unchanged parent accounted for nearly all the total circulating radioactivity.

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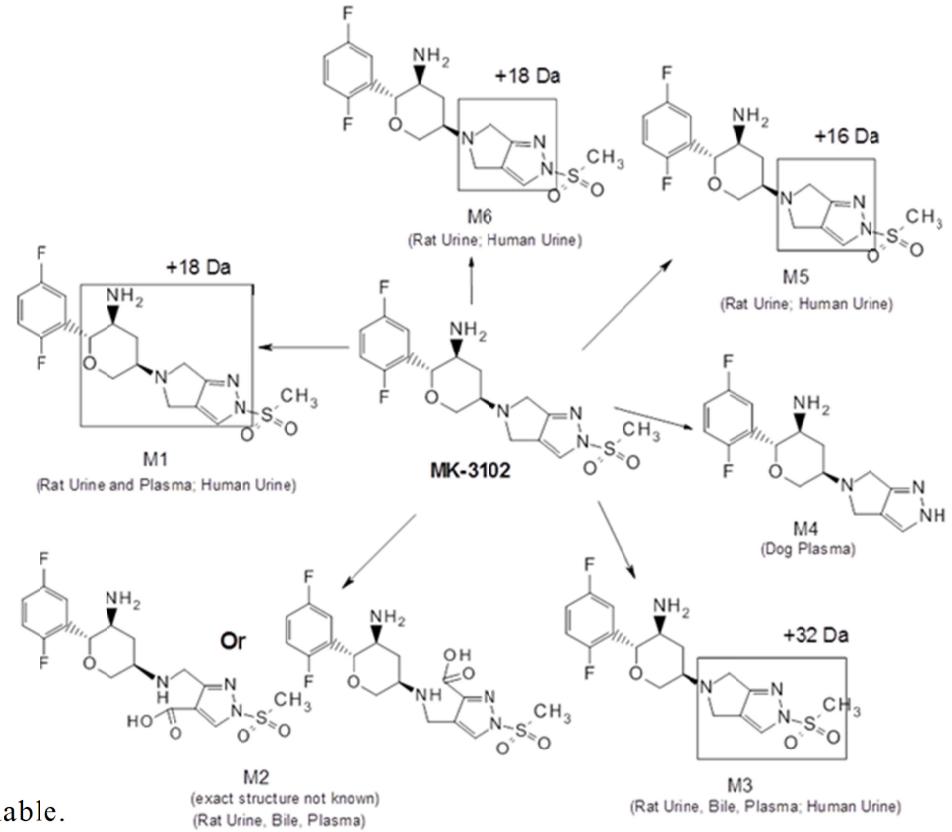
2.6.5.10 推定代謝経路

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK009]
[資料 4.2.2.5: PK003]
[資料 4.2.2.5: PK007]
[資料 4.2.2.5: PK014]



Synthetic standard of M4 is available.

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2.6.5.11 In vitro 代謝

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

Type of Studies: Experiments were conducted to examine the in vitro metabolism of [^{3}H]MK-3102 in liver microsomes from mouse, rabbit, rat, dog, and human, and in hepatocytes from rat, dog, monkey and human.

Source of Materials: Mouse, rabbit, rat, dog, monkey and human liver microsomes were prepared at MRL or purchased from [redacted]. Rat, dog, monkey and human cryopreserved hepatocytes were purchased from [redacted]

Methods: [^{3}H]MK-3102 (1 and 10 μM) was incubated with mouse, rabbit, rat, dog, monkey and human liver microsomes (1 mg/mL microsomal protein) at 37°C in 0.1 M potassium phosphate buffer (pH 7.4) containing 1 mM $MgCl_2$ Reactions were initiated by the addition of NADPH (final concentration 1 mM) and terminated by the addition of equal volumes of acetonitrile. Samples were then vortexed, centrifuged, and the supernatants were analyzed directly.

[^{3}H]MK-3102 (1 and 10 μM) was incubated with rat, dog, monkey and human hepatocytes (1×10^{6} cells/mL) at 37°C for 2 hr in Hepatocyte incubation media purchased from [redacted]. Reactions were terminated by the addition equal volumes of acetonitrile. Samples were then vortexed, centrifuged, and the supernatants were analyzed.

Assays: LC-MS/MS with radiochemical detection.

Additional Information: The in vitro metabolism of [^{3}H]MK-3102 (1 and 10 μM) was studied in CD-1 mouse, Dutch-Belted rabbit, Sprague Dawley rat, beagle dog, rhesus monkey, and human liver microsomes after 60-min incubations in the presence of NADPH. Metabolic turnover of MK-3102 was not observed in these in vitro incubations.

The metabolism of [^{3}H]MK-3102 (1 and 10 μM) was studied in Sprague Dawley rat, beagle dog, rhesus monkey, and human hepatocytes after 2-hr incubation. Metabolic turnover of MK-3102 was not observed in these in vitro incubations.

C Confidential

2.6.5.12 In vitro 代謝（ヒト CYP 分子種の同定）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

Types of Studies: Experiments were carried out to determine the cytochrome P450 (CYP) isoforms that catalyze the oxidative metabolism of [^{3}H] MK-3102.

Methods: [^{3}H]MK-3102 (1 μM) was incubated with microsomes from cells containing recombinant P450 isoforms, including CYP1A2, CYP2A6, CYP3A4, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP2E1, with the same incubation conditions as liver microsomal incubations.

Additional Information: No metabolic turnover was observed when [^{3}H]MK-3102 (1 μM) was incubated with a variety of human recombinant CYP isoforms, including CYP1A2, CYP2A6, CYP3A4, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP2E1. Experiments with inhibitory antibodies against specific CYP enzymes were not feasible due to the lack of turnover in human liver microsomes.

C Confidential

2.6.5.13 ラットにおけるマスバランス

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.5: PK003]

Table 1

Species:	Rat
Gender/Number of Animals:	M/4
Feeding Condition:	Fasted
Vehicle/Formulation:	Saline
Method of Administration:	PO
Dose (mg/kg):	2
Radionuclide:	^{3}H
Specific Activity (μCi/mg):	198
Analyte:	Total radioactivity
Assays:	LSC

C Confidential

2.6.5.13 ラットにおけるマスバランス（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.5: PK003]

Table 2　Excretion of Radioactivity in Bile Duct-Cannulated Rats Dosed Orally With [^{3}H]MK-3102 at 2 mg/kg[a]

Matrix and Time Interval	2 mg/kg PO (n=4) (% of Radioactive Dose)	Matrix and Time Interval	2 mg/kg PO (n=4) (% of Radioactive Dose)	Matrix and Time Interval	2 mg/kg PO (n=4) (% of Radioactive Dose)
Bile		Urine		Feces	
0 to 4 hr	3.7 ± 0.3				
4 to 8 hr	3.2 ± 0.9	0 to 8 hr	22 ± 3.0		
8 to 24 hr	6.0 ± 2.5	8 to 24 hr	17 ± 2.9	0 to 24 hr	1.9 ± 1.1
24 to 48 hr	3.3 ± 0.8	24 to 48 hr	12 ± 2.3	24 to 48 hr	1.8 ± 1.0
48 to 72 hr	0.7 ± 0.4	48 to 72 hr	2.8 ± 0.9	48 to 72 hr	0.8 ± 0.4
72 to 96 hr	0.2 ± 0.1	72 to 96 hr	1.0 ± 0.3	72 to 96 hr	0.3 ± 0.1
96-120 hr	0.1 ± 0.0	96 to 120 hr	0.5 ± 0.3	96 to 120 hr	0.2 ± 0.0
Total	17 ± 2.8	Total	55 ± 4.0	Total	5.0 ± 1.7
Total Recovered	77 ± 0.4				

[a] Bile duct-cannulated male Sprague-Dawley rats were dosed orally with a solution of [^{3}H]MK-3102 in saline. Bile, urine and feces were collected at regular intervals. Radioactivity in bile, urine and cage wash was determined by direct liquid scintillation counting; fecal samples were solubilized prior to liquid scintillation counting. Values shown are mean ± standard deviation values (n=4).

Additional Information: Following oral administration of [^{3}H]MK-3102 to bile duct-cannulated rats at 2 mg/kg, 55%, 17%, and 5% of the dose was recovered in urine, bile, and feces, respectively, up to 120-hr post-dose. About 54% of the dose was recovered in the first 24 hr. Parent compound represented ~86% and 19% of the total radioactivity in urine and bile, respectively, corresponding to ~61% and ~4.2% of the recovered dose. The renal clearance of unbound drug was calculated to be 4.5 mL/min/kg, by dividing the mean total plasma clearance (1.1 mL/min/kg) by the fraction unbound in plasma (0.15), and multiplying the fraction of the dose excreted unchanged into urine (~0.61) [2.6.5.3 項] [2.6.5.6 項] [2.6.5.9 項].

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2.6.5.14 イヌにおけるマスバランス

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.5: PK007]

Table 1

Species:	Dog
Gender/Number of Animals:	M/3
Feeding Condition:	Fasted
Vehicle/Formulation:	0.5% methylcellulose
Method of Administration:	PO
Dose (mg/kg):	2
Radionuclide:	^{14}C
Specific Activity (μCi/mg):	7
Analyte:	Total radioactivity
Assays:	LSC

C Confidential

2.6.5.14 イヌにおけるマスバランス（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.5: PK007]

Table 2　Excretion of Radioactivity in Bile Duct-Cannulated Dogs Dosed Orally With [^{14}C]MK-3102 at 2 mg/kg[a]

Matrix and Time Interval	2 mg/kg PO (n=3) (% of Radioactive Dose)	Matrix and Time Interval	2 mg/kg PO (n=3) (% of Radioactive Dose		
	Bile		Urine	Feces	Cage Wash
0 to 8 hr	1.5±0.3				
8 to 24 hr	2.1±0.6	0 to 24 hr	18±11	0.3±0.1	0.5±0.5
24 to 30 hr	0.4±0	24 to 48 hr	27±8.1	3.0±2.0	0.6±0.2
30 to 48 hr	0.7±0.3	48 to 72 hr	11±3.3	1.3±0.3	0.3±0.1
48 to 54 hr	0.2±0	72 to 96 hr	3.0±1.5	1.5±0.8	0.1±0.0
54 to 72 hr	0.2±0.1	96 to 120 hr	0.8±0.3	1.0±0.3	0.04±0.0
Total	5.1±1.3	Total	60±4.5	7.0±1.1	1.5±0.2
Total Recovered	73±4.7				

[a] Bile duct-cannulated male beagle dogs were dosed orally with a solution of [^{14}C]MK-3102 in 0.5% aqueous methylcellulose. Bile, urine and feces were collected at regular intervals. Radioactivity in bile, urine and cage wash was determined by direct liquid scintillation counting; fecal samples were solubilized prior to liquid scintillation counting. Values shown are mean ± standard deviation values (n=3).

Additional Information: Following oral administration of [^{14}C]MK-3102 to bile duct-cannulated dogs at 2 mg/kg, 60%, 5.1%, 7.0%, and 1.5% of the dose was recovered in urine, bile, feces, and cage wash respectively. The collection of bile was 0-72 hr, and urine, feces, and cage wash were collected over 0-120 hr. About 22% of the dose was recovered in the first 24 hr. The renal clearance of unbound drug was calculated to be 1.7 mL/min/kg, by dividing the mean total plasma clearance (0.9 mL/min/kg) by the fraction unbound in plasma (0.43), and multiplying the fraction of the dose excreted unchanged into urine (~0.82) [2.6.5.3 項] [2.6.5.6 項] [2.6.5.9 項].

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2.6.5.15 ヒトにおけるマスバランス

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.5: PK014]

Table 1

Species:	Human
Gender/Number of Animals:	M/6
Feeding Condition:	Fasted
Vehicle/Formulation:	Aqueous solution containing citric acid, denatonium benzoate and orange flavor
Method of Administration:	PO
Dose (mg/kg):	25 mg
Radionuclide:	^{14}C
Specific Activity (μCi/mg):	0.084
Analyte:	Total radioactivity
Assays:	AMS

C Confidential

2.6.5.15 ヒトにおけるマスバランス（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.5: PK014]

Table 2　Cumulative Excretion of Radioactivity in Human Urine and Feces Following an Oral Dose of [^{14}C]MK-3102[a]

Urine	Percent of Dose							
Time[b]/ Subject	0001	0002	0003	0004	0005	0006	Mean	SD[c]
0-4 hr	7.09	8.38	8.36	5.54	7.83	7.35		
4-8 hr	12.9	16.0	13.9	12.0	15.8	13.2		
8-12 hr	20.2	19.7	NS	19.1	22.6	18.9		
12-24 hr	35.0	33.6	30.4	32.0	38.4	28.1		
24-48 hr	50.2	49.2	44.1	48.9	57.7	48.1		
48-72 hr	57.8	55.7	50.6	58.2	67.6	56.4		
72-96 hr	62.4	60.2	53.9	64.6	73.5	65.0		
96-120 hr	66.0	63.0	56.5	68.0	77.3	68.4		
120-144 hr	68.7	64.9	58.4	70.5	79.4	70.5		
144-168 hr	70.4	66.3	60.1	72.2	80.8	71.6		
168-192 hr	71.1	67.0	61.2	73.3	81.6	72.2		
192-216 hr	71.9	67.5	62.2	74.2	82.1	72.7		
216-240 hr	72.4	67.9	63.0	74.8	82.6	73.0		
240-264 hr	72.8	68.1	63.8	75.3	82.9	73.2		
264-288 hr	73.2	68.4	64.6	75.7	83.2	73.5		
288-312 hr	73.5	68.6	65.2	75.9	83.4	73.7		
312-336 hr	73.7	68.7	65.8	76.2	83.6	73.9		
336-360 hr	73.9	68.8	66.2	76.4	83.8	74.0		
360-384 hr	74.0	68.9	66.7	76.5	83.9	74.1		
384-408 hr	74.2	69.0	67.0	76.7	84.0	74.2		
408-432 hr	74.2	69.0	67.4	76.8	84.1	74.3		
432-456 hr			67.7	76.9	84.2	74.4		
456-480 hr			67.7	76.9	84.2	74.5		
Total Urine	74.2	69.0	67.7	76.9	84.2	74.5	74.4	5.9

C Confidential

2.6.5.15 ヒトにおけるマスバランス（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.5: PK014]

Table 2 (Cont.)　Cumulative Excretion of Radioactivity in Human Urine and Feces Following an Oral Dose of [^{14}C]MK-3102[a]

Feces	Percent of Dose							
Time[b]/ Subject	0001	0002	0003	0004	0005	0006	Mean	SD[c]
0-24 hr	0	0.58	0.11	0.10	0.13	0.03		
24-48 hr	0.78	NS	NS	1.05	0.14	NS		
48-72 hr	1.65	NS	1.07	NS	0.15	NS		
72-96 hr	NS	2.17	NS	2.16	0.86	2.55		
96-120 hr	2.14	2.73	2.52	2.71	NS	3.30		
120-144 hr	2.36	NS	NS	NS	2.28	NS		
144-168 hr	2.47	3.12	2.96	3.09	NS	3.46		
168-192 hr	2.54	3.22	NS	NS	2.68	NS		
192-216 hr	2.66	NS	NS	3.36	2.77	3.60		
216-240 hr	NS	3.30	3.17	3.49	NS	3.66		
240-264 hr	2.72	NS	3.28	3.57	2.84	NS		
264-288 hr	2.75	NS	NS	3.63	NS	3.71		
288-312 hr	2.77	3.35	3.42	3.69	2.88	NS		
312-336 hr	NS	NS	NS	NS	NS	NS		
336-360 hr	2.78	NS	NS	3.70	NS	3.74		
360-384 hr	2.79	3.37	NS	NS	2.90	3.76		
384-408 hr	2.80	NS	3.50	3.72	NS	NS		
408-432 hr	NS	NS	3.52	NS	2.92	3.79		
432-456 hr			3.54	3.75	NS	NS		
456-480 hr			NS	3.76	NS	3.80		
Total Feces	2.80	3.37	3.54	3.76	2.92	3.80	3.4	0.4
Total	77.0	72.4	71.2	80.7	87.1	78.3	77.8	5.8

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2.6.5.15 ヒトにおけるマスバランス（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.5: PK014]

Table 2 (Cont.)　Cumulative Excretion of Radioactivity in Human Urine and Feces Following an Oral Dose of [^{14}C]MK-3102[a]

[a] Six healthy volunteers received 25 mg/~2.5 μCi of [^{14}C]MK-3102 in oral solution (on-site formulation). Radioactivity in fecal homogenates and urine was determined by AMS and expressed as percent of administered dose.
[b] Subjects 0001 and 0002 were discharged from the study on Day 18 post-dose, and the other four subjects (0003, 0004, 0005 and 0006) were discharged on Day 20 post-dose.
[c] SD = standard deviation.
NS: No sample collected.

Additional Information: Following oral administration of [^{14}C]MK-3102 (25 mg/~2.5 μCi) to humans, the majority of the administered dose was recovered in urine (74.4% of the dose), with a smaller percentage recovered in feces (3.4% of the dose). The total mean recovery of radioactivity was 77.8%.

C Confidential

2.6.5.16 ラットにおける乳汁中移行

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: TT[redacted]7130]

Excretion Into Milk

Species:	Rat
Lactation Day/Number of Animals:	Day 14/4 animals per dose
Vehicle/Formulation:	0.5% (w/v) methylcellulose in water
Method of Administration:	Oral gavage
Dose (mg/kg/day):	2 and 10 mg/kg/day
Analyte:	MK-3102
Assay:	LC-MS/MS

Analyte:	MK-3102	
Dose (mg/kg/day)	2	10
Lactation day	Day 14	Day 14
Maternal Plasma Conc. (µM)[a]		
2 hour	4.31 ± 0.232	21.0 ± 2.36
Maternal Milk Conc. (µM)[a]		
2 hour	3.22 ± 0.113	16.7± 0.976
Maternal Milk/Plasma Ratio[b]		
2 hour	0.755 ± 0.0636	0.812 ± 0.0629

[a] Mean ± SEM calculated using all individual plasma concentrations.
[b] Values are the Mean ± SEM of the individual milk concentration divided by the corresponding maternal plasma concentration.

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2.6.5.16 ラットにおける乳汁中移行

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.3: TT#[redacted]7130]

Additional Information: MK-3102 was orally administered to pregnant rats from gestation days (GD) 6 through Lactation Day (LD) 14 at doses of 2 or 10 mg/kg/day (n=4 rats/dose), and MK-3102 was detected in the maternal plasma and milk at 2 hr postdose on LD 14. Blood samples were collected from the orbital sinus into EDTA-treated tubes. As soon as possible after blood collection, each female was injected with oxytocin intramuscularly. Approximately 5 minutes later, milk samples were collected by aspiration over a 10-minute period. All rats survived until scheduled study termination. Lactational transfer of MK-3102 at 2 hr postdose at 2 mg/kg/day and 10 mg/kg/day was approximately 75.5% and 81.2%, respectively. This indicated that the concentration of MK-3102 in milk is similar to the maternal plasma concentrations of MK-3102.

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2.6.5.17 ヒト肝ミクロソーム中のチトクロム P450の阻害

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

Types of Studies: Experiments were carried out to evaluate the potential of MK-3102 as reversible inhibitors of human liver microsomal cytochrome P450s (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4).

Methods: The incubation mixtures (0.2 mL final volume) contained microsomal protein (0.25 mg/mL), 100 mM potassium phosphate buffer (pH 7.4), and 3.14 mM $MgCl_2$. The reaction was initiated with an NADPH regenerating system, which had been pre-incubated at 37°C for 15 minutes prior to use, and was allowed to proceed for 3 to 20 min (37°C in a shaking water bath). The reactions were terminated with the addition of methanol or 50% acetonitrile/2.5% formic acid/0.1% methanol (v/v/v, in water) containing an appropriate internal standard and the samples were vortexed and centrifuged. Samples of the CYP2C8 reaction mixtures were diluted with 90% water/10% acetonitrile/0.5%formic acid (v/v, in water), prior to centrifugation. The supernatants were analyzed by LC-MS/MS.

Assay: LC-MS/MS.

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2.6.5.17 ヒト肝ミクロソーム中のチトクロム P450の阻害（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

Table 1　Effect of MK-3102 on Cytochrome P450 Marker Enzyme Activities in Pooled Human Liver Microsomes (Reversible Inhibition)

CYP	Reaction	Absolute IC_{50} (μM)[a]	
		Control Inhibitor	MK-3102
1A2	Phenacetin *O*-Deethylation	0.0069 α-Naphthoflavone	>100 (0%)[b]
2B6	Bupropion Hydroxylation	0.86 Ticlopidine	>100 (0%)
2C8	Amodiaquine *N*-Deethylation	0.15 Montelukast	>100 (10 ± 3.1%)
2C9	Diclofenac 4′-Hydroxylation	0.77 Sulfaphenazole	>100 (0%)
2C19	*S*-Mephenytoin 4′-Hydroxylation	0.21 Benzylnirvanol	>100 (9.2 ± 1.4%)
2D6	Dextromethorphan *O*-Demethylation	0.089 Quinidine	>100 (5.3 ± 1.7%)
3A4	Midazolam 1′-Hydroxylation	0.032 Ketoconazole	>100 (11 ± 6.0%)
3A4	Testosterone 6β-Hydroxylation	0.026 Ketoconazole	>100 (6.9 ± 1.1%)

[a] The absolute IC_{50} is defined as the inhibitor concentration that yields 50% of the mean control activity.
[b] Values in parentheses represent the percent inhibition (mean ± standard deviation) observed at 100 μM.

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2.6.5.17ヒト肝ミクロソーム中のチトクロム P450の阻害（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

Additional Information: The reversible inhibitory effects of MK-3102 on human liver microsomal activity for CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 –mediated reaction were evaluated in this study. MK-3102 is not a reversible inhibitor of these CYP enzymes, with IC_{50} values > 100 μM.

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2.6.5.18 ヒト肝ミクロソーム中 CYP3A4活性の時間依存的阻害

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

Types of Studies: Experiments were carried out to evaluate MK-3102 as potential preincubation time-dependent inhibitors of CYP3A4.

Methods: Pooled human liver microsomes (1 mg/mL) were preincubated at 37°C with 10 and 50 µM of MK-3102 in 100 mM potassium phosphate buffer (pH 7.4) with 1 mM EDTA, 6 mM $MgCl_2$, and an NADPH-generating system for a duration ranging from 5 to 30 min. The incubation mixtures were diluted 10-fold with the same buffer containing 250 µM testosterone and an NADPH-generating system. The incubation was continued for an additional 10 min to monitor the extent of testosterone 6β-hydroxylation. The reactions were terminated by adding 0.4 mL of 75/25, acetonitrile/water (v/v) containing 0.05% formic acid. This termination solution also contained the internal standard for each assay. The samples were spun in a centrifuge at 3,000 x g for 10 min at 4°C, and 0.2 mL aliquots of the supernatants were transferred to new tubes which contained 0.1 mL of 0.05% formic acid in water. The resulting samples were subjected to LC-MS/MS analysis.

The first order rate constants (k_{obs}) for inactivation at 10 and 50 µM were calculated from the negative slope of the lines by linear regression analysis of the natural logarithm of the remaining activity as a function of time using KaleidaGraph Synergy Software (Reading, PA).

Assay: LC-MS/MS

Table 1　Time-Dependent Inhibition of Testosterone 6β-Hydroxylation by MK-3102 in Human Liver Microsomes

MK-3102 (µM)	Rate Constant (min^{-1})
0	0.003
10	0.002
50	0.003

Additional Information: MK-3102 at 10 and 50 µM did not cause time-dependent inhibition of CYP3A4 activity, with rate constant of 0.002 and 0.003 min^{-1}, respectively, compared to 0.003 min^{-1} for solvent control and 0.081 min^{-1} for the positive control, mifepristone (10 µM).

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2.6.5.19 ヒト肝細胞を用いた CYP3A4、1A2及び2B6の誘導

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

[資料 4.2.2.6: PK010]

Types of Studies: Experiments were carried out to evaluate the potential of MK-3102 to induce CYP3A4, CYP1A2 and CYP2B6 mRNA and activity in human hepatocytes.

Methods: The hepatocytes were treated for 48 hr with vehicle control 0.1% (v/v) DMSO, MK-3102 (0.1–20 μM), or the positive control inducer rifampicin (10 μM), omeprazole (50 μM) or phenobarbital (1000 μM) prepared in incubation medium with all solutions being replaced with fresh solutions at 24 hours. At the end of the 48 hr incubation, whole cell-based CYP3A4, CYP1A2 or CYP2B6 enzyme activity was determined using HPLC-MS/MS, and total RNA was isolated for quantitative PCR analysis of CYP3A4, CYP1A2 or CYP2B6 mRNA expression. Changes in the measured responses following treatment with test compounds were reported as fold changes (response to test compound relative to vehicle control) and as percent positive control.

Assays: RT-PCR and LC-MS/MS.

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2.6.5.19 ヒト肝細胞を用いた CYP3A4、1A2及び2B6の誘導（続き）

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.4: PK002]

[資料 4.2.2.6: PK010]

Table 1　Effect of MK-3102 on CYP3A4 mRNA Level and Activity in Cryopreserved Human Hepatocytes

		[redacted]				[redacted]				[redacted]			
		hCYP3A4 mRNA		6β-hydroxylase Activity		hCYP3A4 mRNA		6β-hydroxylase Activity		hCYP3A4 mRNA		6β-hydroxylase Activity	
Compound (μM)	Dose (μM)	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]
RIF	10	34.8	100.0	18.4	100.0	19.8	100.0	10.7	100.0	13.3	100.0	12.5	100.0
MK-3102	0.1	1.2	0.6	0.8	n.r.	1.4	2.1	1.2	1.6	0.8	n r.	0.9	n.r.
	0.5	1.2	0.6	0.7	n.r.	1.4	2.2	1.3	2.8	0.7	n r.	0.9	n.r.
	1	1.6	1.8	0.6	n.r.	1.4	2.0	1.1	1.1	0.9	n.r.	0.8	n.r.
	5	1.5	1.6	0.6	n.r.	1.7	3.7	1.3	2.8	1.0	0.3	0.8	n.r.
	10	1.4	1.3	0.8	n.r.	1.4	2.0	1.3	3.1	0.9	n r.	0.9	n.r.
	20	1.6	1.7	0.9	n.r.	1.5	2.4	1.3	2.8	0.9	n r.	1.0	0.4

[a] Fold – represents the mean fold change of treated samples compared to vehicle control samples.
[b] % PC – represents the percent of induction relative to positive control rifampicin (10 μM) corrected for vehicle control.
n.r. = not reported since response was less than vehicle control.

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2.6.5.19 ヒト肝細胞を用いた CYP3A4、1A2及び2B6の誘導（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.4: PK002]
[資料 4.2.2.6: PK010]

Table 2　Effect of MK-3102 on CYP1A2 mRNA Level and Activity in Cryopreserved Human Hepatocytes

		[redacted]				[redacted]				[redacted]			
		hCYP1A2 mRNA		O-deethylation Activity		hCYP1A2 mRNA		O-deethylation Activity		hCYP1A2 mRNA		O-deethylation Activity	
Compound (μM)	Dose (μM)	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]
OM	50	106.4	100.0	21.1	100.0	48.6	100.0	11.6	100.0	48.6	100.0	21.8	100.0
MK-3102	0.1	1.1	0.3	0.9	n.r	1.0	n r	1.0	n.r	0.9	n r	1.1	0.4
	0.5	1.2	0.2	1.0	0.2	1.0	n r	1.2	1.6	0.8	n r	1.2	0.9
	1	1.3	0.6	1.0	0.1	1.3	0.5	1.0	0.1	1.3	0.6	1.1	0.6
	5	1.5	0.6	0.9	n.r	1.4	0.8	1.3	2.9	1.3	0.7	1.1	0.5
	10	1.5	0.5	1.3	1.5	1.2	0.5	1.1	0.8	1.3	0.6	1.4	1.9
	20	1.6	0.7	1.4	1.9	1.2	0.5	1.6	5.4	1.3	0.7	1.6	2.7

[a] Fold – represents the mean fold change of treated samples compared to DMSO vehicle control samples.
[b] % PC – represents the percent of induction relative to positive control omeprazole (50 μM) corrected for vehicle control.
n.r. - not reported since response was less than vehicle control.

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2.6.5.19 ヒト肝細胞を用いた CYP3A4、1A2及び2B6の誘導（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.4: PK002]
[資料 4.2.2.6: PK010]

Table 3　The Effect of MK-3102 on CYP2B6 mRNA and Activity in Cryopreserved Human Hepatocytes

		Lot [redacted]				Lot [redacted]				Lot [redacted]			
Treatment	[μM]	hCYP2B6 mRNA		Bupropion hydroxylation		hCYP2B6 mRNA		Bupropion hydroxylation		hCYP2B6 mRNA		Bupropion hydroxylation	
		Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]	Fold[a]	% of PC[b]
PB	1000	15.3	100.0	10.4	100.0	14.2	100.0	12.7	100.0	27.9	100.0	12.9	100.0
MK-3102	0.1	0.9	nr	1.2	2.3	1.0	nr	1.0	nr	0.8	nr	1.0	0.4
	0.5	0.9	nr	1.3	3.7	1.2	1.2	1.0	nr	0.9	nr	1.0	nr
	1	0.9	nr	1.3	3.1	0.8	nr	1.1	0.5	0.8	nr	1.0	nr
	5	1.1	1.0	1.3	3.5	1.8	5.8	1.2	1.7	1.1	0.5	1.0	0.0
	10	1.3	2.3	1.3	3.1	1.9	6.9	1.2	1.8	1.1	0.5	1.0	nr
	20	1.1	0.6	1.1	0.7	1.6	4.4	1.3	2.6	1.0	0.1	1.0	0.2

[a] Fold – represents the mean fold change of treated samples (test article) compared to DMSO vehicle control samples (n=3) or the mean fold change of positive control compared to PBS vehicle control samples (n=3).
[b] % PC – represents the percent of induction relative to positive control phenobarbital (1000 μM) corrected for vehicle control.
nr – not reported since response was less than vehicle control

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2.6.5.19 ヒト肝細胞を用いた CYP3A4、1A2及び2B6の誘導（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.4: PK002]
[資料 4.2.2.6: PK010]

Additional Information: At the concentrations tested (0.1 to 20 μM), MK-3102 did not show significant induction of human CYP3A4 mRNA expression or CYP3A4 mediated 6β-hydroxylation testosterone activity in three hepatocyte preparations (<5% of the response elicited by 10 μM rifampicin). At same concentration range, MK-3102 did not show significant induction of human CYP1A2 mRNA expression or CYP1A2 mediated phenacetin O-deethylation activity in three hepatocyte preparations (<10% of the response elicited by omeprazole). Similarly, MK-3102 did not show significant induction of human CYP2B6 mRNA expression or CYP2B6 mediated bupropion hydroxylation activity in three hepatocyte preparations (<5% of the response elicited by phenobarbital). The positive controls, rifampicin (10 μM), omeprazole (50 μM), and phenobarbital (1000 μM) significantly induced mRNA levels and activities of CYP3A4, CYP1A2, and CYP2B6, respectively, in all hepatocyte preparations.

C Confidential

2.6.5.20 In vitro でのヒト UGT1A1及び SULT1E1阻害

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.6: PK008]

Types of Studies: Experiments were carried out to determine the inhibitory effects of MK-3102 on UGT1A1-mediated estradiol 3-glucuronidation in human liver microsomes and SULT1E1-mediated ethinyl estradiol 3-sulfation in human liver cytosol

Method: Pooled human liver microsomes (0.5 mg/mL) was incubated with 20 µM estradiol, a substrate for UGT1A1, 5 mM UDPGA and 25 µg/mL alamethicin in 200 µL of 81 mM HEPES and 9 mM $MgCl_2$ buffer, pH 7.0 at 37°C for 20 minutes. Estradiol glucuronidation was quantified in the absence (solvent control) and presence of increasing concentrations (0.78-100 µM) of either MK-3102 or nicardipine, a known inhibitor of UGT1A1. The reactions were terminated by adding 0.2 mL of ice-cold methanol containing labetolol as the internal standard. The samples were spun in a centrifuge at 4,000 x g for 30 min at 4°C. The resulting samples were subjected to LC-MS/MS analysis

Pooled human liver cytosol (0.05 mg/mL) was incubated with 8 nM [^{3}H] ethinyl estradiol, 50 µM PAPS, 5 mM $MgCl_2$ in 500 µL of potassium phosphate buffer (0.1 mM, pH 7.4) at 37°C for 10 minutes. Ethinyl estradiol sulfation was quantified in the absence (solvent control) and presence of increasing concentrations of either MK-3102 (0.78-100 µM) or quercetin (0.039-5 µM), a known inhibitor of SULT1E1-mediated ethinyl estradiol sulfation (Schrag ML et al. 2004). The resultant supernatants were dried under a nitrogen stream, and the residues were reconstituted in water:acetonitrile:methanol (1:1:1, by volume). The resulting samples were subjected to LC-MS/MS analysis

The IC_{50} values were calculated by nonlinear regression analysis. (KaleidaGraph by Synergy Software).

Table 1　Summary of IC_{50} Values of MK-3102 as Inhibitors of Human UGT1A1 and SULT1E1

Enzyme	Substrate	Positive Control Inhibitor	MK-3102
UGT1A1	Estradiol (20 µM)	Nicardipine (IC_{50}: 2.54 µM)	>100 µM
SULT1E1	Ethinyl estradiol (0.008 µM)	Quercetin (IC_{50}: 0.6 µM)	>100 µM

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2.6.5.20 In vitro でのヒト UGT1A1及び SULT1E1阻害（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.6: PK008]

Additional Information: The inhibitory potential of MK-3102 on UGT1A1-mediated estradiol 3-glucuronidation was evaluated in human liver microsomes supplemented with UDPGA. MK-3102 did not inhibit UGT1A1-mediated estradiol 3-glucuronidation in human liver microsomes (IC_{50} >100 μM). In contrast, the positive control inhibitor nicardipine potently inhibited the UGT1A1 activity (IC_{50} = 2.54 μM).

The inhibitory potential of MK-3102 on ethinyl estradiol 3-sulfation was evaluated in human liver cytosol supplemented with PAPS. MK-3102 did not inhibit ethinyl estradiol 3-sulfation in human liver cytosol (IC_{50} >100 μM). In contrast, the positive control inhibitor, quercetin potently inhibited the sulfation activity (IC_{50} = 0.6 μM), similar to what was reported in the literature (Schrag ML et al. 2004).

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2.6.5.21 MDR1 P-糖蛋白、BCRP、MATE1、OATP1B1及び OATP1B3を介した輸送に与える影響

Test Article: MK-3102

Location in CTD/Study No.:[資料 4.2.2.6: PK004]
[資料 4.2.2.6: PK012]
[資料 4.2.2.6: PK015]

Types of Studies: Experiments were carried out to evaluate MK-3102 as a potential inhibitor of MDR1 P-glycoprotein, BCRP, OATP1B1, OATP1B3 -mediated transport activity.

Methods: The LLC-MDR1 and LLC-PK1 cell lines were cultured in 24-well transwell culture plates (Millipore, Billerica, MA). [^{3}H]Digoxin (0.1 μM) and the inhibitors were prepared in transport buffer (Hanks buffer with 10 mM HEPES, pH 7.4). MK-3102 was tested at 0-300 μM. Prior to each transport study, cells were washed twice with transport buffer. Substrate solution (500 μL) was added to either the A or B compartment of the culture plate, and buffer (500 μL) was added to the compartment opposite to that containing the compound. The inhibitor at various concentrations was added to both compartments. At t=1.5 hr, samples were taken out from both sides and scintillant (UltimaFlo-M, Perkin Elmer, Boston, MA) was added. Radioactivity was determined by liquid scintillation counting in a Beckman LS6500 scintillation counter (Beckman; Fullerton, CA). The experiment was performed in triplicate.

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2.6.5.21 MDR1 P-糖蛋白、BCRP、MATE1、OATP1B1及び OATP1B3を介した輸送に与える影響（続き）

Test Article: MK-3102
Location in CTD/Study No.:[資料 4.2.2.6: PK004]
[資料 4.2.2.6: PK012]
[資料 4.2.2.6: PK015]

The inhibitory effect of the test compound on ATP-dependent [^{3}H]-methotrexate (MTX) (10 µM, [redacted]) uptake was conducted in membrane vesicles isolated from baculovirus infected Spodoptera frugiperda (Sf9) cells containing human BCRP (ABCG2) ([redacted]). All reagents were commercially obtained. Briefly, 20 µL of [^{3}H]-MTX, with or without various concentrations of test compound or 5 µM KO143 (prototypic BCRP inhibitor), dissolved in transport buffer (0.25 M sucrose, 10 mM Tris-HCl buffer (pH 7.4), 10 mM MgCl2) was added to 10 µL (2 mg protein/mL) of BCRP vesicles and pre-incubated for 5 minutes at 37°C. Uptake was initiated by the addition of 20 µL ATP regenerating reagent or AMP reagent (final concentration of 5 mM ATP or AMP, 10 mM creatine phosphate and 100 µg/mL creatine phosphokinase in transport buffer), followed by incubation at 37°C for the indicated time. Uptake was stopped by the addition of 200 µL ice-cold stop buffer (0.25 M sucrose, 0.1 M NaCl, 10 mM Tris-HCl buffer (pH 7.4)), followed by transfer of the reaction mixture to pre-wetted 96-well glass fiber type B filter plate (1.0 µm) (Millipore, Billerica, MA), and application of vacuum. Filters containing the membrane vesicles were washed with 200 µL ice-cold stop buffer, six times. The filter plate was dried and 50 µL scintillation fluid (Optiphase Supermix, Perkin Elmer, Boston, MA) was added to each sample. Radioactivity was determined by liquid scintillation counting in a MicroBeta Wallac Trilux Scintillation Counter (Perkin Elmer, Boston, MA). The experiment was performed in triplicate. ATP-dependent [^{3}H]-MTX uptake in hBCRP containing vesicles was calculated by subtracting the uptake in the absence of ATP from that in the presence and data were then normalized to % control, where uptake in the absence of test compound was 100%.

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2.6.5.21 MDR1 P-糖蛋白、BCRP、MATE1、OATP1B1及び OATP1B3を介した輸送に与える影響（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.6: PK004]
[資料 4.2.2.6: PK012]
[資料 4.2.2.6: PK015]

The inhibitory effects of the test compound on MATE1-mediated [^{14}C] metformin (5 µM, [redacted]) uptake were conducted in MATE1 stably transfected CHO-K1 cells (CHO-K1-MATE1) and control CHO-K1 cells. CHO-K1 and CHO-K1-MATE1 cells were obtained from [redacted] ([redacted]) and were used under license agreement. All other reagents were commercially obtained. Cells were dislodged with trypsin EDTA (Invitrogen) and resuspended in Hanks' Balanced Salt Solution (HBSS) with 10 mM Hepes, pH 7.4. Cells were then suspended in 96-deep well plates (BD Falcon, San Jose, CA) at a density of 0.4 x 10^6 cells/well in 125 µL of HBSS. Uptake was initiated by the addition of 125 µL of [^{14}C] metformin containing various concentrations of the test compound or quinidine (a prototypical MATE1 inhibitor) dissolved in DMSO at 2X the final concentration tested (5 µM [^{14}C] metformin and 100 µM quinidine final concentration). Cells were then incubated for 10 minutes at 37°C in a temperature controlled shaker, and uptake was stopped by the addition of ice-cold phosphate buffered saline (PBS), immediate centrifugation for 2 minutes at 3000 rpm at 4ºC (Eppendorf, Model 5180R; Hamburg, Germany), followed by washing of the cell pellets with PBS four times. Cell pellets were lysed in 20 µL 20% acetonitrile (Sigma-Aldrich) and 200 µL scintillation fluid was added (Ultima Gold; Perkin Elmer, Boston MA). Radioactivity was determined by liquid scintillation counting in a MicroBeta Wallac Trilux Scintillation Counter (Perkin Elmer, Boston MA). The experiment was performed in triplicate. MATE1-mediated metformin uptake was calculated by subtracting the uptake of metformin into CHO-K1 cells from that observed in CHO-K1-MATE1 cells and data were normalized to % control, where uptake in the absence of test compound was 100%.

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2.6.5.21 MDR1 P-糖蛋白、BCRP、MATE1、OATP1B1及び OATP1B3を介した輸送に与える影響（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.6: PK004]
[資料 4.2.2.6: PK012]
[資料 4.2.2.6: PK015]

The inhibitory effect of MK-3102 on OATP1B1-mediated [^{3}H] pitavastatin uptake and OATP1B3-mediated [^{3}H] sulfobromophthalein (BSP, 0.1 µM, synthesized by [redacted]) uptake was conducted in MDCKII cells stably transfected with an OATP1B1 cDNA (MDCKII-OATP1B1) and an OATP1B3 cDNA (MDCKII-OATP1B3), respectively. Cells were treated with 10 mM sodium butyrate (Sigma-Aldrich, St. Louis, MO) 24 hours prior to the experiment to increase expression of OATP1B1/1B3. Cells were dislodged with trypsin EDTA (Invitrogen) and resuspended in HBSS with 10 mM Hepes, pH 7.4. Cells were then suspended in 96-deep well plates (BD Falcon, San Jose, CA) at a density of 0.4 x 10^6 cells/well. Uptake was initiated by the addition of 0.1 µM [^{3}H] Pitavastatin or BSP (final concentration) containing various concentrations of the test compound or 10 µM cyclosporin A (prototypic inhibitor) dissolved in HBSS buffer with 10 mM Hepes, pH 7.4. Cells were then incubated for 5 minutes at 37°C and uptake was stopped by the addition of ice cold phosphate buffered saline (PBS), immediate centrifugation for 2 minutes at 3000 rpm at 4ºC (Eppendorf, Model 5180R; Hamburg, Germany), followed by washing of the cell pellets with PBS, 4 times. Cell pellets were re-suspended in 50% acetonitrile (Sigma-Aldrich) and scintillation fluid was added (Scintisafe Econo 2; Fisher Chemicals, Pittsburgh, PA). Radioactivity was determined by liquid scintillation counting in a LS6500 Multipurpose Scintillation Counter (Beckman Coulter, Brea, CA). The experiment was performed in triplicate. OATP1B1-mediated Pitavastatin uptake was calculated by subtracting the uptake of Pitavastatin into MDCKII cells from that observed in the OATP1B1 transfected cells. Similarly, OATP1B3-mediated BSP uptake were calculated by subtracting the uptake of BSP into MDCKII cells from that observed in the OATP1B3 transfected cells. Data were normalized to % control, where uptake in the absence of test compound was 100%.

Assays: Liquid Scintillation Counter.

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2.6.5.21 MDR1 P-糖蛋白、BCRP、MATE1、OATP1B1及び OATP1B3を介した輸送に与える影響（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.6: PK004]
[資料 4.2.2.6: PK012]
[資料 4.2.2.6: PK015]

Table 1　Effect of MK-3102 on the Bi-Directional Transport of [^{3}H]-Digoxin (0.1 µM) in LLC-PK1 and LLC-MDR1 Cell Monolayers

Inhibitor Concentration (µM)	B-A/A-B Ratio		% Net Transport	Inhibition % of Control
	LLC-PK1	LLC-MDR1		
No inhibitor	2.3	17.7	5.53	100.0
0.3	Not Determined	13.2	4.88	88.4
1		16.1	4.63	83.7
3		16.3	4.84	87.6
10		14.2	5.02	90.9
30		16.7	6.47	117.1
100		15.7	6.19	112.0
300		12.4	6.44	116.5
Positive Control 10 µM Cyclosporine		1.3	0.67	12.1

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2.6.5.21 MDR1 P-糖蛋白、BCRP、MATE1、OATP1B1及び OATP1B3を介した輸送に与える影響（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.6: PK004]
[資料 4.2.2.6: PK012]
[資料 4.2.2.6: PK015]

Table 2　Summary of IC_{50} Values of MK-3102 as Inhibitors of Human BCRP, MATE1, OATP1B1, and OATP1B3

Transporter	Substrate	Positive Control Inhibitor	MK-3102
BCRP	Methotrexate (10 μM)	KO143 (5 μM)	>100 μM
MATE1	Metformin (5 μM)	Quinidine (100 μM)	> 50 μM
OATP1B1	Pitavastatin (0.1 μM)	Cyclosporine A (10 μM)	>100 μM
OATP1B3	BSP (0.1 μM)	Cyclosporine A (10 μM)	>100 μM

Additional Information: The effect of MK-3102 on human MDR1 P-glycoprotein (P-gp)-mediated efflux transport of digoxin was evaluated using bidirectional transport studies in LLC-PK1 cells and LLC-PK1 cells stably expressing human MDR1 P-gp cDNA (LLC-MDR1 cells). MK-3102 showed no inhibition (< 20% decrease in net transport) of MDR1 P-gp-mediated digoxin transport in LLC-MDR1 cells over the concentration range tested (0.3 - 300 μM). An IC_{50} value was not calculated.

MK-3102 did not significantly inhibit BCRP-mediated [^{3}H] methotrexate uptake up to 100 μM, maximum concentration tested and thus the IC_{50} >100 μM. MK-3102 did not significantly inhibit MATE1-mediated [14C] metformin uptake up to 50 μM, maximum concentration tested, thus the IC50 >50 μM. MK-3102 did not significantly inhibit OATP1B1-mediated [^{3}H] Pitavastatin uptake up to 100 μM, maximum concentration tested and thus the IC_{50} >100 μM. MK-3102 inhibited 14% of OATP1B3-mediated [^{3}H] BSP uptake at 100 μM, maximum concentration tested and thus the IC_{50} >100 μM.

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2.6.5.22 hOAT1、hOAT3、hOCT1及び hOCT2を介した輸送の基質及び阻害剤としての評価

Test Article: MK-3102

Location in CTD/Study No.: [資料 4.2.2.6: PK005]

Types of Studies: Experiments were carried out to evaluate MK-3102 as substrate and potential inhibitor of OAT1, OAT3, OCT1 and OCT2-mediated transport activity.

Method: hOAT1- and hOAT3-mediated [^{14}C]MK-3102 (1 μM) uptake were determined in MDCKII cells stably transfected with hOAT1 (MDCKII-hOAT1 cells) and hOAT3 cDNAs (MDCKII-hOAT3 cells), respectively. Uptake was initiated by the addition of [^{14}C] MK-3102 (1 μM) or positive control substrates [^{3}H] PAH (1 μM) and [^{3}H] ES (1 μM) for hOAT1 and hOAT3, respectively. Cells were incubated for the indicated time at 37°C, then uptake was stopped by the addition of ice cold phosphate buffered saline (PBS, Invitrogen), followed by immediate centrifugation for 1 minute at 3000 rpm at 4ºC (Eppendorf, Model 5180R; Hamburg, Germany) and washing of the cell pellets with PBS for three times. Cell pellets were resuspended in 50% acetonitrile (Sigma-Aldrich) and the radioactivity was determined by liquid scintillation counting in a LS6500 Multipurpose Scintillation Counter (Beckman Coulter, CA). The experiment was performed in triplicate.

hOCT1 and hOCT2-mediated MK-3102 (1 μM) uptake were determined in hOCT1 transiently transfected CHO-K1 cells (CHO-K1-hOCT1 cells) and in hOCT2 stably transfected CHO-K1 cells (CHO-K1-hOCT2 cells), respectively. Uptake was initiated by the addition of MK-3102 (1 μM) or positive control substrate [^{14}C]TEA (1 μM) dissolved in HBSS buffer. Cells were incubated for the indicated time at 37°C, followed by the washing procedure described above. Cells pellets incubated with [^{14}C]TEA were either resuspended in 50% acetonitrile in the hOCT1 assay or detached with 1% Triton X-100 (Fisher Chemicals, Pittsburgh, PA) in the hOCT2 assay, and radioactivity was determined by liquid scintillation counting in a LS6500 Multipurpose Scintillation Counter (Beckman Coulter, CA). Whereas cell pellets incubated with MK-3102 were resuspended in 50% acetonitrile containing labetolol (Sigma-Aldrich) as internal standard, and samples were analyzed quantitatively by LC/MS/MS.

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2.6.5.22 hOAT1、hOAT3、hOCT1及び hOCT2を介した輸送の基質及び阻害剤としての評価（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.6: PK005]

The inhibitory effect of MK-3102 on hOAT1-mediated [^{3}H] cidofovir (1 μM), hOAT3-mediated [^{3}H] cimetidine (1 μM) and hOCT1-mediated [^{14}C] metformin (1 μM) uptake was conducted in MDCKII-hOAT1, MDCKII-hOAT3, and CHO-K1-hOCT1, respectively. Except for CHO-K1-hOCT1 cells, 24 hours prior to the experiment, cells were treated with 10 mM sodium butyrate (Sigma-Aldrich) to increase hOAT1 and hOAT3 expression. Cells were dislodged with trypsin EDTA (Invitrogen) and resuspended in HBSS with 10 mM Hepes (pH 7.4). Cells were then suspended in 96-deep well plates (BD Falcon) at a density of 0.6 x 10^{6} cells/well, 0.4 x 10^{6} cells/well and 0.4 x 10^{6} cells/well for hOAT1, hOAT3, and hOCT1 inhibition assays, respectively. Uptake was initiated by the addition of 1 μM [^{3}H] cidofovir, 1 μM [^{3}H] cimetidine or 1 μM [^{14}C] metformin containing various concentrations of MK-3102 dissolved in HBSS buffer (+10 mM Hepes, pH 7.4). Cells were then incubated for 10 minutes at 37°C, followed by the washing procedure and sample preparation described in the uptake assay in MDCKII-hOAT1 and MDCKII-hOAT3 cells.

The inhibitory effect of MK-3102 on hOCT2-mediated [^{14}C] metformin (1 μM) uptake was conducted in CHO-K1-hOCT2 cells. Cells were prepared as described in the uptake assay in CHO-K1-hOCT2 cells. Uptake was initiated by the addition of 1 μM [^{14}C] metformin containing various concentrations of MK-3102 dissolved in HBSS buffer (+10 mM Hepes, pH 7.4). Cells were then incubated for 10 minutes at 37°C and uptake was stopped by the addition of ice cold phosphate buffered saline (PBS), followed by washing of the cells with PBS for three times. Cells were detached with 1% Triton X-100 (Fisher Chemicals, Pittsburgh, PA) and scintillation fluid was added (Scintisafe Econo 2; Fisher Chemicals). Radioactivity was determined by liquid scintillation counting in a LS6500 Multipurpose Scintillation Counter (Beckman Coulter, CA).

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2.6.5.22 hOAT1、hOAT3、hOCT1及び hOCT2を介した輸送の基質及び阻害剤としての評価（続き）

Test Article: MK-3102
Location in CTD/Study No.: [資料 4.2.2.6: PK005]

Table 1　Summary of IC_{50} Values of MK-3102 as Inhibitors of Human OAT1, OAT3, OCT1 and OCT2

Transporter	Substrate	Positive Control Inhibitor	MK-3102
OAT1	Cidofovir (1 μM)	Probenecid (1mM)	>100 μM
OAT3	Cimetidine (1 μM)	Probenecid (1 mM)	55.9 μM
OCT1	Metformin (1 μM)	Quinidine (5 μM)	>50 μM
OCT2	Metformin (1 μM)	Quinidine (100 μM)	>50 μM

Additional Information: No active uptake of MK-3102 was observed in cells expressing hOCT1, hOCT2, hOAT1, or hOAT3. In contrast, active uptake was observed with tetraethylammonium (a model substrate of hOCT1 and hOCT2), p-aminohippurate (a model substrate of hOAT1), and estrone sulfate (a model substrate of hOAT3), thus confirming the functionality of the expression systems used for hOCT1, hOCT2, hOAT1, and hOAT3. Under the conditions tested, MK-3102 is not a substrate for hOAT1, hOAT3, hOCT1 and hOCT2.

MK-3102 (1-100 μM) did not inhibit hOAT1-mediated [^{3}H] cidofovir (1 μM) uptake. Probenecid, a known inhibitor for organic anion transporters, significantly inhibited [^{3}H] cidofovir (1 μM) uptake.

MK-3102 inhibited hOAT3-mediated [^{3}H] cimetidine (1 μM) uptake, with an IC_{50} value of 55.9 ± 8.1 μM. Probenecid, a known inhibitor for organic anion transporters, significantly inhibited [^{3}H] cimetidine (1 μM) uptake.

MK-3102 (up to 50 μM) did not significantly inhibit hOCT1-mediated [^{14}C] metformin (1 μM) uptake (IC_{50} >50 μM). Quinidine (at 5 μM), a known inhibitor for organic cation transporters, inhibited [^{14}C] metformin (1 μM) uptake.

MK-3102 (0.1 - 50 μM) did not inhibit hOCT2-mediated [^{14}C] metformin (1 μM) uptake. Quinidine, a known inhibitor for organic cation transporters, inhibited [^{14}C] metformin (1 μM) uptake.

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