CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:
21-272

PHARMACOLOGY REVIEW

NDA 21-272

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

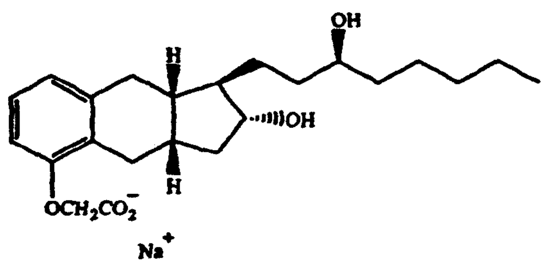
Xavier Joseph, D.V.M.
March 12, 2001

ORIGINAL NDA DATED: August 11, 2000
CENTER RECEIPT DATE: August 14, 2000
REVIEWER RECEIPT DATE: August 15, 2000

SPONSOR: United Therapeutics Corp.
P.O.Box 14186, Research Triangle Park, NC 27709

DRUG PRODUCT: Remodulin Injection

DRUG: Generic name – Treprostinol sodium
Code names – UT-15, 15AU81 and LRX-15



M.W. 412.49

FORMULATION: Remodulin Injection is a sterile sodium salt solution supplied in 20 ml multi-use vials containing 1.0, 2.5, 5.0 or 10.0 mg/ml of treprostinol. Each ml of the formulation also contains 5.3 mg sodium chloride (except for the 10.0 mg/ml concentration which contains 4.0 mg sodium chloride), 3.0 mg metacresol, and 6.3 mg sodium citrate. Sodium hydroxide and hydro-chloric acid are added to adjust the pH between 6.0 and 7.2.

PHARMACOLOGICAL CLASS: Prostacyclin (PGI_2) analog

PROPOSED INDICATION: Treatment of pulmonary arterial hypertension (PAH)

PROPOSED DOSAGE REGIMEN: Remodulin is administered by continuous subcutaneous infusion at an initial infusion rate ≤1.25 ng/kg/min, with upward and downward adjustments based on PAH symptoms and drug-related adverse effects. It is recommended that increments not exceed 1.25 ng/kg/min per week for the first four weeks and 2.5 ng/kg/min per week for the remaining duration of infusion.

IND UNDER WHICH CLINICAL TRIALS WERE CONDUCTED: [redacted]

TABLE OF CONTENTS

SUMMARY OF PHARMACODYNAMIC STUDIES

(The pharmacological data submitted in this new drug application are compiled from studies conducted at the [redacted]

UT-15 (formerly known as 15AU81 or LRX-15), a chemically stable tricyclic benzindene analogue of prostacyclin (PGI_2, epoprostenol), is currently being developed for the treatment of pulmonary arterial hypertension. In vitro and in vivo pharmacodynamic studies have shown that UT-15 possesses systemic and pulmonary vasodilatory and platelet antiaggregatory properties. These studies are summarized below.

In Vitro Studies Related to Proposed Indication

1. Platelet Antiaggregatory Activity

Rat platelet-rich plasma was incubated with UT-15 at concentrations ranging from 5.1 to 102.4 nM (2-40 ng/ml) for 1 minute at 37°C prior to the addition of ADP (10 μM). UT-15 produced a concentration-dependent inhibition of ADP-induced platelet aggregation (Table 1) with an IC_{50} of 34.6 nM (13.5 ng/ml)

Table 1.

INHIBITION OF PLATELET AGGREGATION IN VITRO IN RAT PLATELET-RICH PLASMA BY UT-15

Concentration (nM)	% Inhibition
5.1	8 ± 5
10.2	12 ± 5
25.6	27 ± 8
51.2	81 ± 3
102.4	100 ± 0

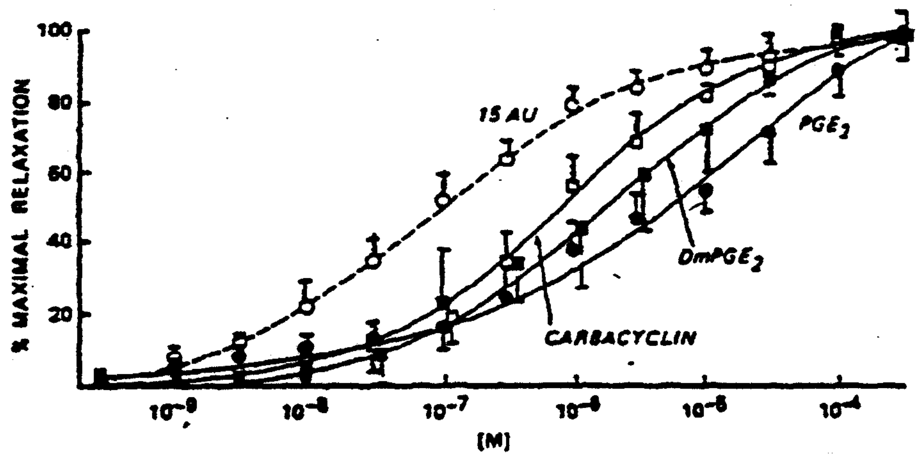
Results, expressed as % inhibition of control aggregation, are mean ± S.E.M. of 3 experiments.

In vitro studies using human platelet-rich plasma showed that UT-15 [2.56 to 256 nM (1-100 ng/ml] also caused a concentration-dependent inhibition of ADP-induced aggregation of human platelets, with an IC_{50} of 28.2 nM (11 ng/ml). It is stated that UT-15 was found to be 20-fold less potent than prostacyclin in inhibiting the ADP-induced aggregation of human platelets.

2. Vascular Relaxation Effect

UT-15 (1-1000 nM) produced a concentration-dependent relaxation of isolated rabbit mesenteric artery segments precontracted with the thromboxane mimetic U-46619 (1 µM; Figure 1), the order of potency (when compared to other prostaglandins) being UT-15 > carbacyclin (a stable prostacyclin analogue) > 16-dimethyl PGE_2 > PGE_2. In this study, UT-15 was found to be about 8 and 45 times more potent in inducing vascular relaxation than carbacyclin and PGE_2, respectively.

Figure 1.



Relaxant actions of PGE_2 (●, n = 6), 15AU81 (O, n = 6), 16-dimethyl PGE_2, $DmPGE_2$ (■, n = 4), and carbacyclin (□, n = 5), in rabbit mesenteric artery precontracted with 10^{-6}M U-46619, a thromboxane mimetic. Results, expressed as % maximum relaxations, are shown as Mean ± SEM.

3. Antiproliferative Effect in Human Pulmonary Artery Smooth Muscle Cells

UT-15 (30 nM for 48 hours) markedly reduced the proliferation of cultured human pulmonary artery smooth muscle cells, as measured by blinded cell counting (92% reduction) and [^{3}H] thymidine incorporation (61% reduction). Moreover, it was shown that UT-15 produced a large elevation (about 120 fold) in intracellular cAMP, which was still elevated (about 6 fold) 72 hours after drug treatment. The above results indicate that UT-15 exerts its antiproliferative effect via a cAMP-dependent pathway in pulmonary artery smooth muscle cells.

In Vivo Studies Related to Proposed Indication

1. Platelet Antiaggregatory Activity

Pentobarbitone anesthetized rats were given subcutaneous or oral administration of UT-15. Blood was collected 20, 40 or 60 min after dosing, centrifuged, and the platelet-rich plasma was used for ADP (10 μm)-induced platelet aggregation studies. UT-15 at 100 μg/kg, sc, caused significant inhibition of platelet aggregation at both 20 and 40-minutes, while 25 μg/kg, sc, did not produce any significant inhibition of platelet aggregation (Table 2). When administered orally, UT-15 produced significant inhibition of ADP-induced platelet aggregation only at 5000 μg/kg (Table 3), but not at lower doses (25, 100 or 1000 μg/kg; Tables 2 and 3.)

(In the above study, UT-15, at 25 and 100 μg/kg sc, reduced mean arterial blood pressure by 29 and 60 mmHg, respectively. Following oral administration, the mean arterial blood pressure was reduced by 35 and 55 mmHg at 1000 and 5000 μg/kg doses, respectively.)

Table 2. Inhibition of Platelet Aggregation Ex Vivo Following Subcutaneous or Oral Administration of UT-15 in the Rat

Dose (μg/kg)	Route	Time (min)	% Inhibition
25	sc	20	7 ± 4
	sc	40	18 ± 6
100	sc	20	71 ± 9 ***
	sc	40	20 ± 1 *
25	po.	20	6 ± 6
	po.	40	1 ± 2
100	po.	20	2 ± 6
	po.	40	7 ± 3

Results, shown as the % inhibition of sub maximal ADP (10 μM)-induced platelet aggregation in rat platelet-rich plasma prepared ex vivo, 20 and 40 min after administration of UT-15, are mean ± S.E.M. of 3-4 experiments for each group. Statistically significant difference from the control aggregation is shown by *P < 0.05; ***P < 0.001.

Table 3. Inhibition of Platelet Aggregation Ex Vivo Following Oral Administration of High Doses of UT-15 in the Rat

Dose (mg/kg)	Time (min)	% Inhibition
1	20	27 ± 17
5	20	58 ± 8 ***
	60	53 ± 23

Results, shown as the % inhibition of submaximal ADP (10 μM) – induced platelet aggregation in rat platelet-rich plasma, prepared ex vivo 20 or 60 min after oral administration of UT-15, are mean ± S.E.M. of 3-4 experiments for each group. Statistically-significant difference from the control aggregation is shown by *P < 0.05; ***P < 0.001.

In pentobarbitone anesthetized rabbits, 10-minute iv infusions of UT-15 (50-500 ng/kg/min) also caused dose-related inhibition of ADP-induced platelet aggregation. In these studies, the ID_{50} (dose causing 50% inhibition of platelet aggregation) for UT-15 was found to be 140 ng/kg/min, as compared to 200 ng/kg/min for prostacyclin. These antiaggregatory doses of UT-15 and prostacyclin produced reductions in mean blood pressure of 10 and 16 mmHg, respectively, indicating only minimal differences between these agents as inhibitors of platelet aggregation or vasodilators in this model.

In an anesthetized open-chest dog model with stenosed circumflex coronary arteries, UT-15 (300-1125 ng/kg/min iv infusion) was found to be 4.3-fold less potent than prostacyclin in preventing platelet thrombus formation and 7-fold less potent in lowering the mean arterial blood pressure.

2. Vasodilator and Hemodynamic Activity

a. Anesthetized Animals

In pentobarbitone-anesthetized rats, UT-15 produced significant dose-related reductions in mean arterial pressure (MAP) when administered by subcutaneous (29-60 mmHg at 25-100 μg/kg) and oral (35-55 mmHg at 1-5 mg/kg) routes. Intravenous infusion of UT at 0.4 μg/kg/min caused a reduction in MAP by 31 mmHg (Table 4). UT-15 was found to be about 10-fold less potent than prostacyclin as a hypotensive agent in this animal model.

Table 4.

HYPOTENSIVE EFFECTS OF UT-15 IN ANESTHETIZED ANIMALS

Species	Dose	Route	BP Change (mm Hg)	n
Rat	25 μg/kg	sc	29 ± 4	4
	50 μg/kg	sc	36 ± 3	4
	100 μg/kg	sc	60 ± 5	5
	0.4 μg/kg/min	iv.	31	--
	1 mg/kg	po.	35 ± 7	6
	5 mg/kg	po.	55 ± 9	3
Rabbit	0.05 μg/kg/min	iv.	8 ± 3	3
	0.1 μg/kg/min	iv.	18 ± 6	3
	0.2 μg/kg/min	iv.	28 ± 9	3
	0.4 μg/kg/min	iv.	48 ± 8	3
	0.5 μg/kg/min	iv.	54 ± 11	3
Cat	3 μg/kg/min	iv.	22 ± 8 (D)	4
	10 μg/kg/min	iv.	36 + 16 (D)	4
	30 μg/kg/min	iv.	74 ± 9 (D)	4
Dog	0.32 μg/kg	iv.	8 ± 2	5
	1 μg/kg	iv.	14 ± 2	5
	3.2 μg/kg	iv.	36 ± 2	5
	0.1 μg/kg/min	iv.	8 + 4	3
	0.3 μg/kg/min	iv.	27 ± 12	3
	1 μg/kg/min	iv.	63 ± 12	3

Results are shown as the reduction in mean systemic arterial blood pressure or diastolic blood pressure (D), expressed as mean ± S.E.M. All blood pressure (BP) changes shown are statistically significant ($P < 0.05$).

APPEARS THIS WAY
ON ORIGINAL

In pentobarbitone-anesthetized rabbits, 10-minute iv infusions of UT-15 at 0.05 to 0.5 µg/kg/min produced dose-related reductions in MAP (8-54 mmHg; Table 4). In rabbits, there was very little difference in potency between UT-15 and prostacyclin as hypotensive agents.

In chloralose-anesthetized cats (closed chest), iv infusion of UT-15 (3-30 µg/kg/min for 20 min) also caused dose-related reductions in diastolic blood pressure (22-74 mmHg; Table 4) accompanied by non-dose related tachycardia (27-30 bpm). The maximum hypotensive and cardiac responses to UT-15 were evident within 5 minutes of infusion, and the values returned to baseline levels within 40 minutes of completing the infusion. No direct comparison of the potency of UT-15 with prostacyclin was done in this study.

In chloralose and urethane mixture-anesthetized open-chest cats, iv infusions of UT-15 (0.1-3.0 µg/kg/min for 20 minutes each) produced dose-dependent decreases in mean systemic arterial (6-42%) and mean pulmonary arterial (2-26%) blood pressures (Table 5). UT-15 did not significantly affect heart rate or cardiac index. UT-15, at these dose levels, produced dose-dependent reductions in hypoxia-induced increments in pulmonary artery pressure (Figure 2) and pulmonary vascular resistance (Figure 3). In this study, UT-15 was about 3 and 10 times less potent than prostacyclin as a vasodilator under hypoxic and normoxic conditions, respectively.

In anesthetized newborn piglets, UT-15 at 6 µg/kg (iv bolus) abolished hypoxia-induced increases in pulmonary vascular resistance.

In pentobarbitone-anesthetized dogs, iv bolus injections (0.32-3.2 µg/kg) or 10 min iv infusions (0.1–1.0 µg/kg/min) of UT-15 produced significant dose-related reductions in MAP (Table 4). In animals that received the iv bolus injection, there was a reduction in MAP of 8 to 36 mmHg, the duration of the hypotensive response varied from 30 seconds at the low dose to several minutes at the high dose. Ten-minute iv infusions of UT-15 caused dose-related reductions in MAP (8-62 mmHg) with significant dose-related reductions in total peripheral resistance (0.2-1.4 units), and significant reduction in LVdP/dt (648 mmHg/ sec) at 1.0 µg/kg/min (Table 6). There were no significant heart rate, cardiac index or EKG findings.

Four hour iv infusions of UT-15 (0.1, 0.3, 1.0 and 3.0 µg/kg/min) in anesthetized dogs produced dose-dependent decreases in MAP (10-68%) and total peripheral resistance (TPR, 20-73%). Although decreases in pulmonary artery pressure (variable) and pulmonary vascular resistance (PVR, 9-33%) were noted, these effects were not dose-related (Figures 4 & 5). The vascular effects were rapid in onset, achieving maximum effect within 5-10 min of infusion with rapid recovery on termination of infusion. Although the effect on PVR was not dose-related, plasma sample analysis showed a close relationship between plasma concentrations of UT-15 and changes in TPR and PVR. (Pharmacodynamic modeling predicted maximum decreases in total peripheral resistance of 66% and pulmonary vascular resistance of 22%.) The plasma concentrations of UT-15 producing 50% of the maximum effect on systemic and pulmonary vascular resistances were 8.6 ng/ml and 11.3 ng/ml, respectively.

In the above dog model, UT-15 produced dose-dependent decreases in left ventricular inotropic (+dP/dt) activity at 1 and 3 μg/kg/min doses, and significant dose-dependent decreases in left ventricular lusitropic (-dP/dt) activity at doses of 0.3 μg/kg/min and above. At 3.0 μg/kg/min, both +dP/dt and – dP/dt exhibited an apparent rebound above control values on termination of infusion. Significant decreases in left ventricular end diastolic pressure were noted at all dose levels (not dose-related). Cardiac output was significantly increased at 0.3 μg/kg/min and above and heart rate was increased at 0.3 and 3.0 μg/kg/min (30-68%). UT-15 produced dose-dependent decreases in PR and QRS intervals with no significant effect on QT_c.

UT-15 infusions produced dose-related increases in plasma angiotensin II concentrations (50-263 pg/ml; Table 7) which correlated inversely with reduction of mean arterial blood pressure.

Prostacyclin (PGI_2), when given to anesthetized dogs for 4 hours at 0.01 to 0.3 μg/kg/min, produced vascular and cardiac effects similar to those produced by UT-15 (Figures 4 & 5); however, PGI_2 was found to be 10 times more potent than UT-15 in these studies. PGI_2 infusions also caused dose-dependent increases in plasma angiotensin II concentrations (Table 8).

A separate study in anesthetized dogs showed that pretreatment with an angiotensin converting enzyme inhibitor, enalapril (0.3 mg/kg, iv), prevented the UT-15-induced increases in plasma angiotensin II concentrations. Digoxin (100 mg/kg) pretreatment attenuated the ability of UT-15 to elevate plasma angiotensin II levels while pretreatment with a loop diuretic (furosemide, 1.0 mg/kg) potentiated the increase in plasma angiotensin II concentrations induced by UT-15. Moreover, the results of this study suggest that pretreatment with enalapril, digoxin or furosemide may enhance the cardiovascular effects of UT-15.

Table 5. RESTING HEMODYNAMIC VARIABLES BEFORE HYPOXIA IN DIFFERENT GROUPS OF ANESTHETIZED CATS

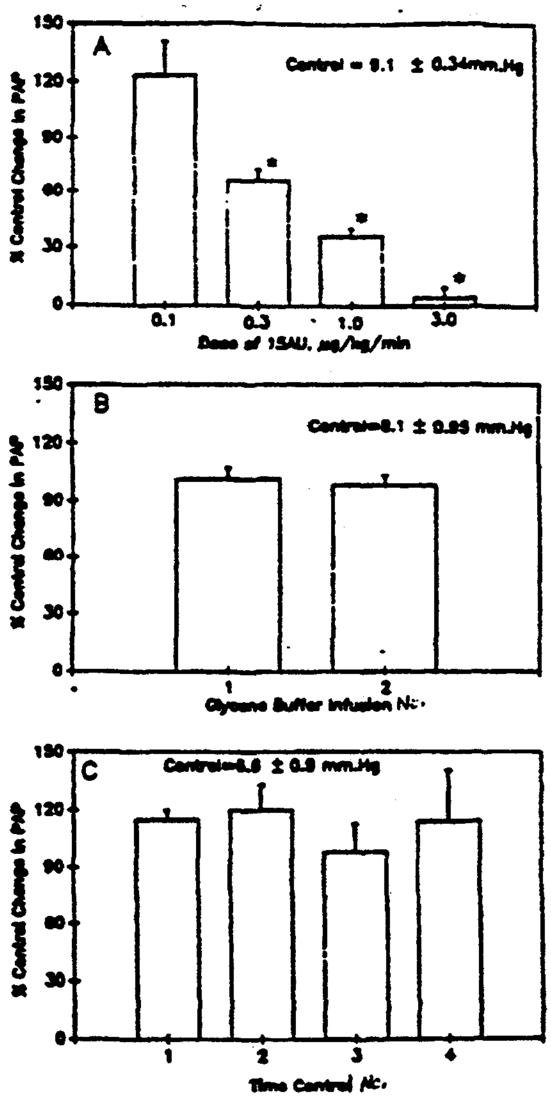
Group	SMAP mmHg	HR bpm	CI ml/min/kg	SVR mmHg/L/min/kg	PAP mmHG	PVP mmHg	PVR mmHg/L/Min/kg
UT-15, N = 5							
Dose, µg/kg/min							
Control	118 ± 3.7	225 ± 9	97 ± 10	1298 ± 146	15.3 ± 1	2.2 ±.0.6	169 ± 24
0.1	111 ± 3.2	228± 13	95 ± 12	1271 ± 147	14.9 ± 1.3	2.3 ± 0.7	167 ± 20
0.3	102 ± 5.5	220 ± 14	91 ± 9	1162 ± 78	13.7 ± 0.6	2.1 ± 0.9	156 ± 13
1.0	88 ± 5.3*	212 ± 14	85 ± 9	1072 ± 68	12.2± 0.9*	2.0 ± 0.7	155 ± 22
3.0	68 ± 12*	211 ± 14	81 ± 10	880 ± 97	11.3 ± 1*	2.1 ± 0.8	146 ± 15
Glycine buffer, N = 5							
Infusion (0.1 ml/min) #							
Control	124 ± 5	255 ± 7	75 ± 8	1720 ± 113	14.7 ± 0.7	2.1 ± 0.2	196 ± 15
1	118 ± 5	244 ± 11	70. ± 8	1769 ± 158	14.2 ± 1.2	2.0 ± 0.4	209 ± 16
2	122 ± 4	249 ± 13	67 ± 10	1971 ± 213	15.7 ± 1	2.0 ± 0.3	253 ± 33

Table 5. RESTING HEMODYNAMIC VARIABLES BEFORE HYPOXIA IN DIFFERENT GROUPS OF ANESTHETIZED CATS (CONTINUED)

Group	SMAP mmHg	HR bpm	CI ml/min/kg	SVR mmHg/L/min/kg	PAP mmHg	PVP mmHg	PVR mmHg/L/Min/kg
Time Control, N = 5							
Time Period #							
Control	100 ± 2	264 ± 13	66 ± 7	1599 ± 148	13.9 ± 1.1	3.3 ± 0.6	233 ± 38
1	101 ± 6	268 ± 11	74 ± 7	1415 ± 141	15.4 ± 1.4	3.2 ± 0.7	216 ± 25
2	94± 6	262 ± 13	61 ± 6	1617 ± 186	14.2 ± 1.2	3.5 ± 0.8	251 ± 36
3	103 ± 4	275 ± 6	64 ± 8	1688 ± 147	16.3 ± 1.3	3.6 ± 0.7	278 ± 40
4	87 ± 8	257 ± 12	57 ± 9 **	1627 ± 221	15.7 ± 1.7	3.0 ± 0.7	309 ± 56

Asterisks indicate significant differences from corresponding control value. *$P < 0.01$, **$P < 0.05$. SMAP = Systemic Mean Arterial Pressure, HR = Heart Rate, CI = Cardiac Index, SVR = Systemic Vascular Resistance, PAP = Mean Pulmonary Arterial Pressure, PVP = Mean Pulmonary Venous Pressure, PVR = Pulmonary Vascular Resistance. Data represent resting values prior to the hypoxic challenges during control period and during each infusion. In time control experiments, data represent values prior to the hypoxic challenges before and at various times after a bolus injection of saline.

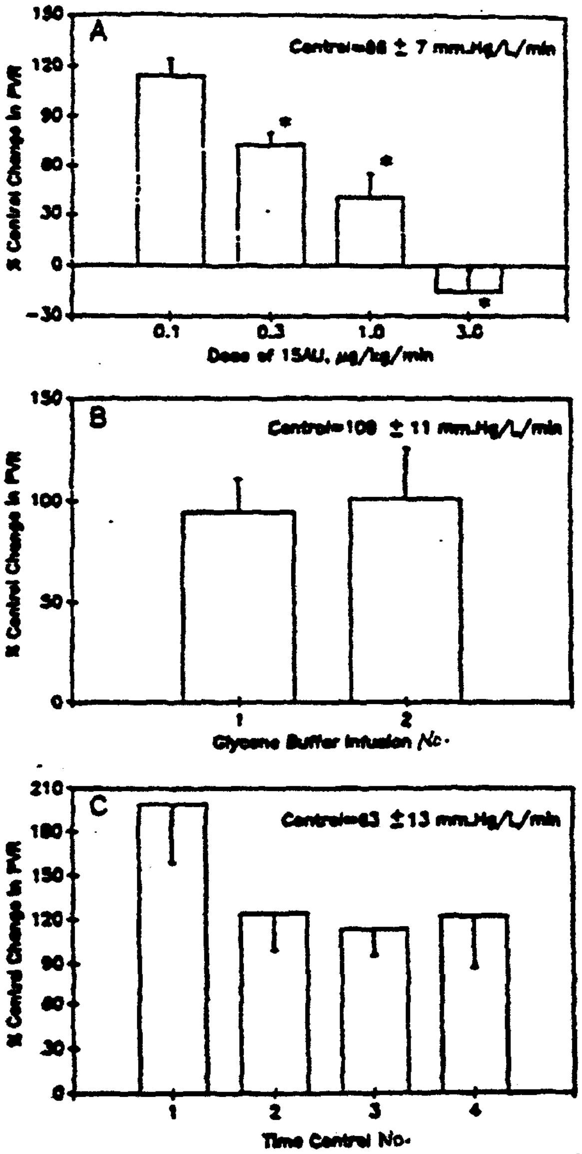
Figure 2.



Effects of 15AU81 on the systemic hypoxia-induced increment in pulmonary arterial blood pressure (PAP) (panel A). Also shown are the effects of glycine buffer (panel B) and responses in time control experiments (panel C). All results (mean ± S.E.) are expressed as percent of control hypoxic response.

* P< 0.005 vs. Control

Figure 3.



Effects of 15AU81 (panel A), and glycine buffer (panel B) on the systemic hypoxia-induced increment in pulmonary vascular resistance (PVR). Responses in time control experiments are shown in panel C. All results (mean ± S.E.) are expressed as percent of control PVR response.

*P < 0.02 vs control.

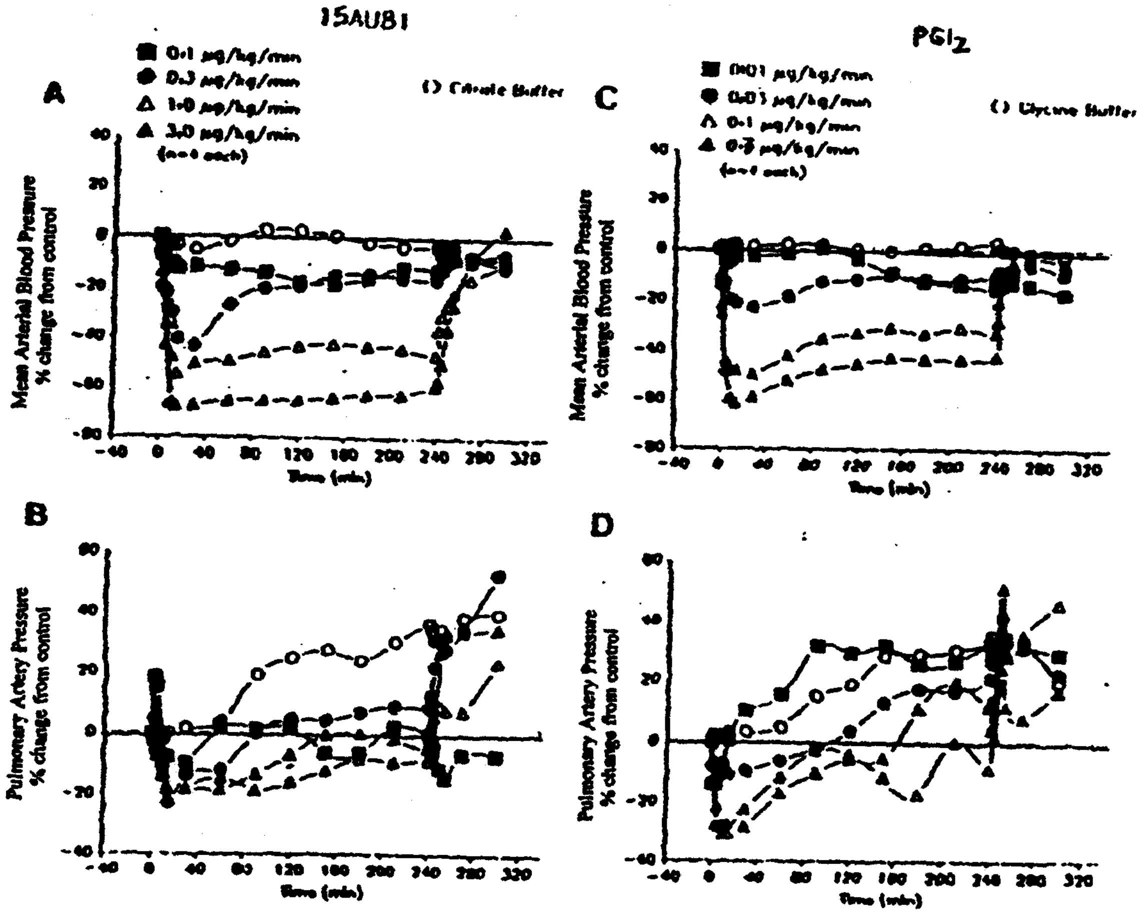
Table 6.

HEMODYNAMIC AND ELECTROCARDIOGRAM EFFECTS OF INTRAVENOUS INFUSIONS OF UT-15 IN THE ANESTHETIZED DOG (N = 3)

	Pre-Infusion Value	UT-15 (µg/kg/min) 0.1	0.3	1.0
Hemodynamic Parameter				
Mean Blood Pressure (mmHg)	136 ± 5	-8 ± 4 *	-27 ± 12*	-62 ± 12*
Total Peripheral Resistance (peripheral resistance units)	3.2 ± 0.5	-0.2 ± 0.4 *	-0.7 ± 0.6*	-1.4 ± 0.5*
Left Ventricular End Diastolic Pressure (mm Hg)	4.2 ± 1.0	0.7 ± 0.8	0.2 ± 1.0	-0.9 ± 1.3
Heart Rate (bpm)	147 ± 12	9.0 ± 8.4	2.0 ± 6.6	-9.0 ± 2.4
Cardiac Index (ml/min/kg)	152 ± 15	6 ± 18	26 ± 40	0.2 ± 2.3
LVdP/dt (mmHg/sec)	2339 ± 404	101 ± 161	-105 ± 113	-648 ± 39*
Electrocardiogram Parameter				
ST-Segment Elevation (mV)	-0.06 ± 0.07	0 ± 0.03	-0.02 ± 0.02	0.0 ± 0.04
PR-interval (msec)	104 ± 2.3	-3.3 ± 2.9	-2.3 ± 5.0	4.7 ± 6.4
QT_c-interval (msec)	321 ± 7.7	12.7 ± 5.2	7.0 ± 2.5	-6.0 ± 2.1

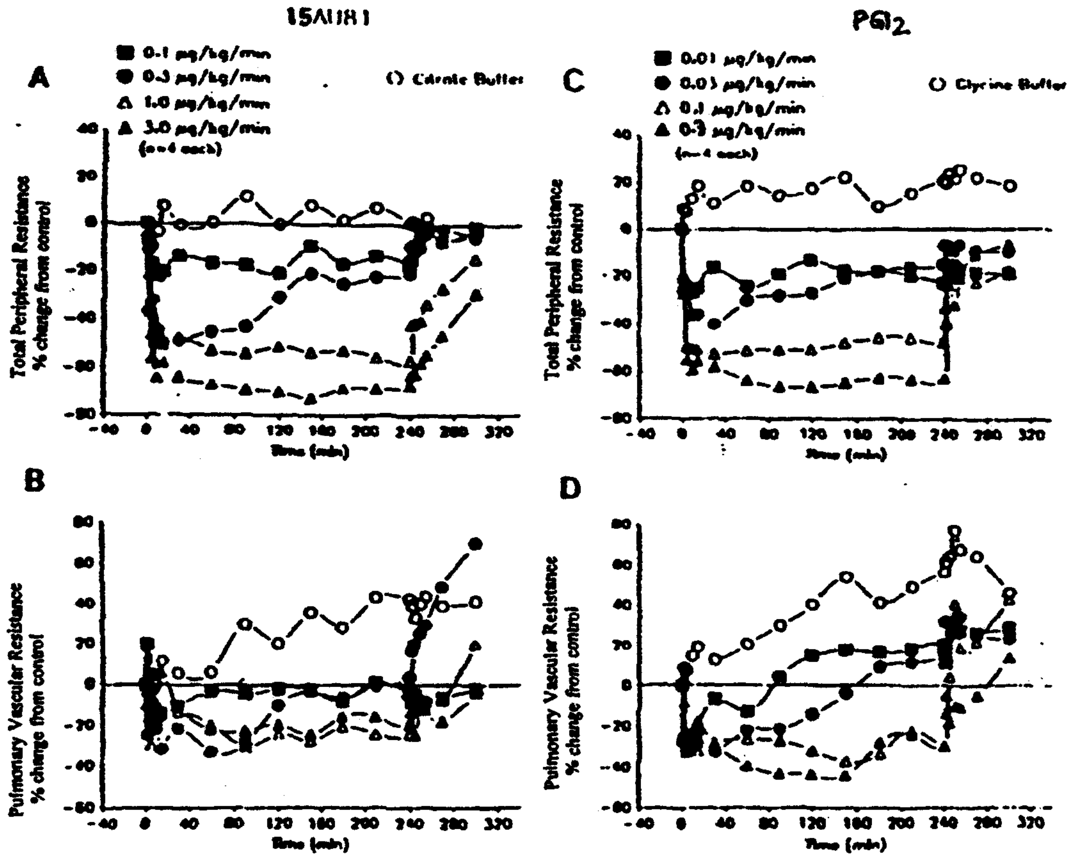
Data, shown as the change from pre-infusion values for each parameter are the mean ± S.E.M. Each dose was infused for 10 minutes. Where there is significant change from pre-infusion value this is shown as *P < 0.05.

FIGURE 4



The percent change from control of mean arterial blood pressure and mean pulmonary artery blood pressure during and 60 minutes following 240 min infusions of 15AU81, Panels A and B, respectively, and PGI_2, Panels C and D, respectively, in pentobarbital anesthetized dogs. Citrate and glycine buffer are included as vehicle controls for 15AU81 and PGI_2, respectively. Four animals per treatment with one treatment per animal.

FIGURE 5



The percent change from control of mean total peripheral resistance and mean pulmonary vascular resistance during and 60 minutes following infusions of 15AU81, Panels A and B, respectively, and PGI_2 Panels C and D, respectively, in pentobarbital anesthetized dogs. Citrate and glycine buffer are included as vehicle controls for 15AU81 and PGI_2 respectively. Four animals per treatment with treatment per animal.

APPEARS THIS WAY
ON ORIGINAL

Table 7.

EFFECT OF UT-15 ON VENOUS PLASMA ANGIOTENSIN II CONCENTRATION (PG/ML) [DIFFERENCE BETWEEN PREINFUSION AND 220 MINUTES OF INFUSION]

15AU81, µg/kg/min	Angiotensin II conc.
0.1 (n = 3)	50.50 ± 42.43
0.3 (n = 4)	68.05 ± 17.23
1.0 (n = 4)	255.53 ± 119.07
3.0 (n = 4)	*263.45 ± 79.15
citrate buffer (n = 4)	37.45 ± 8.21

Mean ± SEM, *$p < 0.05$ vs citrate buffer

Table 8.

EFFECT OF PGI_2 ON VENOUS PLASMA ANGIOTENSIN II CONCENTRATION (PG/ML). [DIFFERENCE BETWEEN PREINFUSION AND 220 MINUTES OF INFUSION]

PGI_2 µg/kg/min	Angiotensin II conc.
0.03 (n = 4)	23.45 ± 4.35
0.10 (n = 3)	44.27 ± 7.94
0.30 (n = 2)	62.45

Mean ± SEM

b. Conscious Animals

In rats, oral administration of UT-15 at 1.5 and 5 mg/kg produced significant reductions in diastolic blood pressure of 13 and 28 mmHg, respectively (Table 9), with little effect on systolic blood pressure. These responses were seen within 10 min of dosing, and a full recovery was seen within 30-60 minutes. Heart rate was significantly increased (53-183 bpm) at the above dosage levels. The heart rate effect was observed within 10 minutes of drug administration and lasted much longer than the blood pressure effect, up to 4 hours at the high dose.

In spontaneously hypertensive rats, oral administration of UT-15 (0.1, 0.3, 1.0 and 3.0 mg/kg) caused decreases in arterial blood pressure (14-18%) and increases in heart rates (20-25%) at 0.3 mg/kg and above. The hypotensive and tachycardiac effects lasted up to 5 hours. There were no significant changes in the above parameters at 0.1 mg/kg.

In dogs, the hypotensive effect of a 0.5 mg/kg po dose of UT-15 lasted up to 120 minutes post-dose, PR-interval was reduced at 30 min post-dose and QT_c-interval increased at 120 min post-dose (Table 10).

Ten-minute iv infusions of UT-15 (0.3, 1.0 and 3.0 µg/kg/min) in dogs produced dose-related reductions of both systolic (18-40 mmHg) and diastolic (13-45 mmHg) blood pressures (Table 9) with increases in heart rates (13-30 bpm; information on dose relationship not provided). These effects were rapid in onset and a full recovery was evident within 5-10 minutes of termination of infusion.

APPEARS THIS WAY
ON ORIGINAL

Table 9.

HYPOTENSIVE EFFECTS OF UT-15 IN THE CONSCIOUS RAT OR DOG

Species	Dose	Route	BP Change (mm Hg)	n
Rat	1.5 mg/kg	p.o	13 ± 2 (D)	6
	5 mg/kg	po.	28 ± 7 (D)	6
	1 mg/kg	po.	10 ± 3	4
	5 mg/kg	po.	19 ± 3	4
Dog	0.3 μg/kg/min	iv.	13 ± 3 (D)	6
	1 μg/kg/min	iv.	35 ± 6 (D)	6
	3 μg/kg/min	iv.	45 ± 4 (D)	6
	0.5 mg/kg	po.	31 (D)	6
	1.5 mg/kg	po.	53 ± 11	3

Results are shown as the reduction in mean systemic arterial blood pressure or diastolic blood pressure (D), expressed as mean ± S.E.M. All blood pressure (BP) changes shown are statistically significant ($P < 0.05$)

Table 10. HEMODYNAMIC AND ELECTROCARDIOGRAM EFFECTS OF ORAL ADMINISTRATION OF UT-15 (0.5 MG/KG) IN THE CONSCIOUS DOG (N = 6)

		Post-Dosing Values					
	Pre-Dose Values	5 Min	30 Min	60 Min	90 Min	120 Min	140 Min
Hemodynamic Parameter							
Systolic Blood Pressure (mm Hg)	149 ± 9	113 ± 7*	120 ± 4*	122 ± 7*	136 ± 6*	124 ± 5*	147 ± 8
Diastolic Blood Pressure (mm Hg)	67 ± 5	44 ± 7*	54 ± 2*	53 ± 5*	57 ± 6*	55 ± 4	70 ± 6
Heart Rate (bpm)	78 ± 8	102 ± 15	101 ± 6	100 ± 14	84 ± 6	79 ± 5	71 ± 4
Electrocardiogram Parameter							
PR-Interval (Msec)	102 ± 3	--	90 ± 3*	95 ± 3	99 ± 3	106 ± 3	105 ± 4
QRS – Interval (Msec)	52 ± 5	--	58 ± 5	55 ± 6	58 ± 5	53 ± 7	52 ± 6
QT_C-Interval (Msec)	231 ± 7	--	230 ± 8	236 ± 10	244 ± 9	246 ± 10*	235 ± 13

Data, shown as the pre- and post-infusion values of each parameter, are the mean ± S.E.M. Where there is a significant difference from pre-dose value it is shown as *$P < 0.05$.

Safety Pharmacology Studies

1. Autonomic Nervous System (ANS): In anesthetized cats, iv infusion of UT-15, at 3-30 μg/kg/min for 20 minutes, had no effect on nictitating membrane contractions induced by cervical sympathetic nerve stimulation, or on bradycardia induced by vagal nerve stimulation, indicating that UT-15 had no effect on either the sympathetic or the parasympathetic systems of the ANS.

2. Respiratory System: UT-15 (10-100 nM) produced weak contractile responses in guinea pig isolated tracheal segments. In precontracted guinea pig tracheal preparations, UT-15 (10^{-8}-10^{-3} M) caused dose-related relaxation, with an ED_{50} of 270 nM. In this study, UT-15 was found to be equipotent with PGE_2 (ED_{50}=220 nM) and about 400 times more potent than the stable prostacyclin analogue, carbacyclin (ED_{50}=100 μM).

In anesthetized cats, iv infusion of UT-15 (3-30 μg/kg/min for 20 minutes) had minimal effects on both respiratory rate and tidal volume except at the high dose, at which increased respiratory rate (10-15 breaths/min) was seen.

3. Gastrointestinal System: UT-15 exhibited weak contractile effects on isolated segments of guinea pig ileum, rat stomach or rat colon. When given orally, UT-15 inhibited GI motility and fluid secretion in the rat small intestine. In rats, the test drug inhibited the ulcer formation induced by indomethacin or ethanol. It is suggested that the anti-ulcer activity of UT-15 may be due to a cytoprotective rather than an antisecretary effect. In rats, pretreatment with a single dose of UT-15 (0.5 and 5 mg/kg, po) reduced the severity of carbon tetrachloride-induced hepatotoxicity. UT-15 (0.03-10 mg/kg, po) had no effect on pentabarbitone metabolism in rats, as measured by sleep duration, indicating a lack of its effect on liver enzyme function.

4. Reproductive System: UT-15 had no significant effect on primate uterine motility in vivo.

5. Other Effects: In the mouse, UT-15 afforded little or no protection against the lethal effects of platelet activating factor.

SUMMARY OF PHARMACOKINETIC STUDIES

Early pharmacokinetic and metabolism studies in dogs and rats (conducted at the) were performed using [^{3}H] UT-15, in which the fate of total tritium was followed. Since this technique was found to be non-specific, a capable of detecting UT-15 levels as low as was later developed. The United Therapeutic Corporation, after licensing UT-15 from the GlaxoWellcome Co., developed a "highly specific and sensitive' assay method that was claimed to be capable of detecting levels as low as of UT-15/ml of plasma. This was the method employed for the evaluation of the toxicokinetic study blood samples.

Absorption Studies

Male and female Sprague-Dawley rats (6/sex) were administered a single oral or iv dose of 200 µg [^{3}H] UT-15/kg. After oral dosing, maximum plasma levels of total radioactivity were reached within 0.5-1.5 hr in males and 2-6 hr in females. The mean apparent oral bioavailability was determined to be about 46% (both sexes). Although the plasma clearance values were consistent between oral and iv dosings, there was an apparent gender difference. The plasma clearance of total [^{3}H] was more rapid in males (441 ml/hr/kg) than in females (274 ml/hr/kg). The distribution half-life after iv dosing was 0.8 hr (both sexes) and the elimination half-lives were 10–14 hr after iv and 4-7 hr after oral administration. The elimination half-life was shorter in males (4-10 hr) than in females (7-14 hr).

Following a single oral dose of 5 mg [^{3}H] UT-15/kg in Sprague-Dawley rats (3/sex), maximum plasma levels of total radioactivity were reached at 0.75 and 2 hr post-dose in males and females, respectively. The mean apparent bioavailability was determined to be 31%, indicating that the oral bioavailability might decrease with increasing dose. The mean plasma clearance values were 440 ml/hr/kg for males and 276 ml/hr/kg for females. The distribution half-lives of radioactivity were 3.5 and 2.1 hr for males and females, respectively. The elimination half-lives could not be estimated because of possible enterohepatic recirculation.

In anesthetized normotensive beagle dogs (2/sex/group) given 4 hr iv infusions of UT-15 at 0, 0.1, 0.3, 1.0 or 3.0 µg/kg/min, plasma concentrations of UT-15 (determined by increased rapidly and reached steady-state levels within 10-15 min from the onset of infusion at all infusion rates. Pharmacokinetic analysis of the data indicated a biphasic decay of UT-15 in plasma with an initial half-life of about 2 min and a terminal half-life of about 20 min. Concen-tration-effect vs time plot indicated a close relationship between plasma drug concen-tration and the onset of hemodynamic effects [decreases in total peripheral resistance (TPR) and pulmonary vascular resistance (PVR)]. In general, decreases in TPR were maintained during all but the 0.3 µg/kg/min infusions (Figure 6), while decreases in PVR were maintained only at the two highest dose levels (1.0 and 3.0 µg/kg/min; Figure 7). Because plasma concentrations of UT-15 did not decrease during any infusions, it is suggested that some tachyphylaxis may have occurred for TPR at the 0.3 μg/kg/min infusion, and for PVR at the 0.1 and 0.3 μg/kg/min infusions.

Upon termination of the infusion, although plasma drug concentrations dropped close to zero levels, the effects on TPR still persisted for about 60 minutes at 1 and 3 μg/kg/min infusion levels (Figure 6). This hysteresis was more readily observed when the mean effect was plotted against the mean concentration of drug at each infusion rate, and points were linked in temporal sequence (Figure 8). The hysteresis is attributed to a delay in the clearance of drug from the active site compared to its clearance from the plasma, and/or due to the presence of active metabolite at the active site. There was no clear hysteresis in the effect of UT-15 on PVR.

When the concentration-effect data were fitted to the E_{max} pharmacodynamic model, it was predicted that the maximum decrease in TPR achievable with UT-15 in anesthetized dogs was 66%, and the concentration of UT-15 producing 50% of the maximum effect (EC_{50}) was 8.6 ng/ml. Similarly, the predicted maximum reduction in PVR was 22% and the EC_{50} was estimated to be 11.3 ng/ml. While the magnitude of the effect of UT-15 on TPR and PVR may be different, the above model suggested that there might be little or no selectivity of UT-15 for the pulmonary or peripheral circulation in the normotensive, anesthetized dog.

In a separate, nonrandomized crossover study, three anesthetized beagle dogs were given bolus doses of UT-15 orally (200 μg/kg), intravenously (20 μg/kg), or intratracheally (20 μg/kg) with or without Exosurf (a synthetic surfactant used clinically for the treatment of neonatal and adult respiratory distress syndrome), treatments separated by at least one week. Intratracheal administration with or without Exosurf produced mean peak plasma concentrations of 11.9 ng/ml and 12.5 ng/ml at 7 min post-dose, with mean bioavailability values of 46 and 88%, respectively. When administered orally, the mean peak plasma concentration of 96.6 ng/ml was obtained 45 min after dosing, and the mean bioavailability was 62%. After iv administration, the highest concentration (85.7 ng/ml) was seen in the first sample (taken 2 min post-dose). The half-life of UT-15 after iv dosing was determined to be 2.8 minutes.

[The results of the above study indicate that intratracheal or oral dosing can provide an alternate means of systemic delivery of UT-15. However, bioavailability data showed high variability.]

In a two-week continuous iv infusion study in dogs (3/sex/group; infusion rates at 0, 0.05, 0.1 or 0.2 μg/kg/min), infusion-phase plasma drug concentrations were below the limit of quantitation at [redacted]. There was no quantifiable amount present in post-infusion samples. The results indicated that the plasma concentrations were linearly related to the dose.

Pharmacokinetic data obtained from all the above studies are summarized in Table 11.

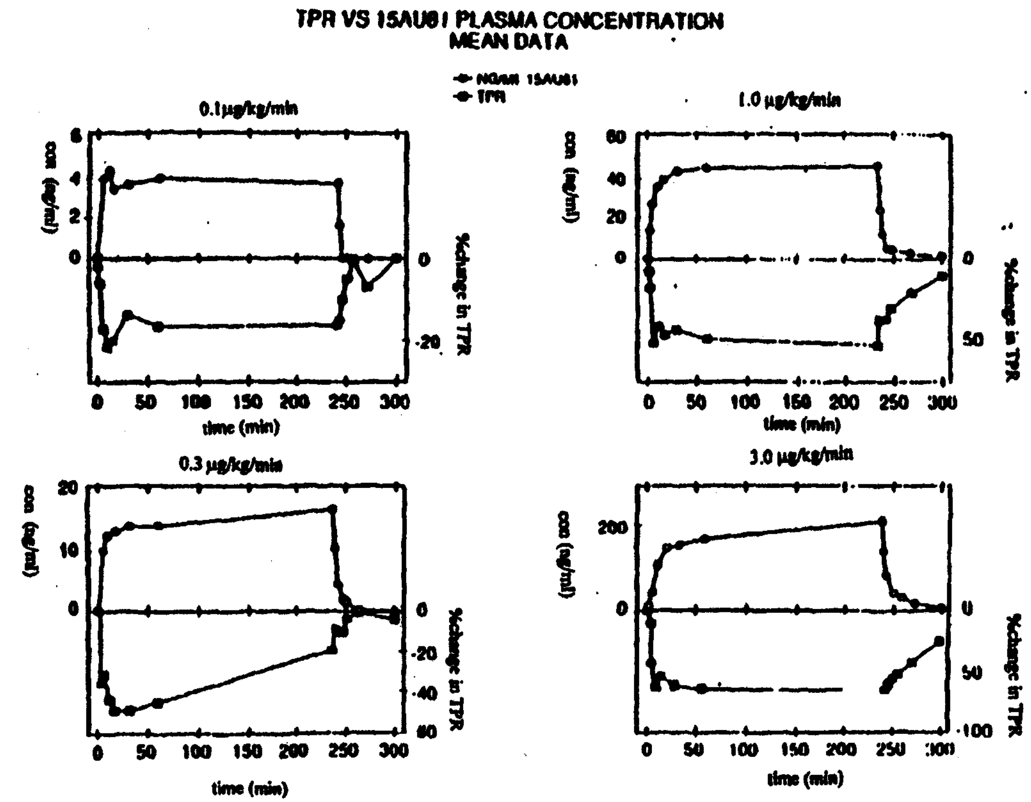


Figure 6. Graphs of mean plasma concentrations of 15AU81 and mean changes in total peripheral resistance (TPR) vs. time, when 15AU81 was infused at 0.1, 0.3, 1.0, and 3.0 µg/kg/min to anesthetized dogs.

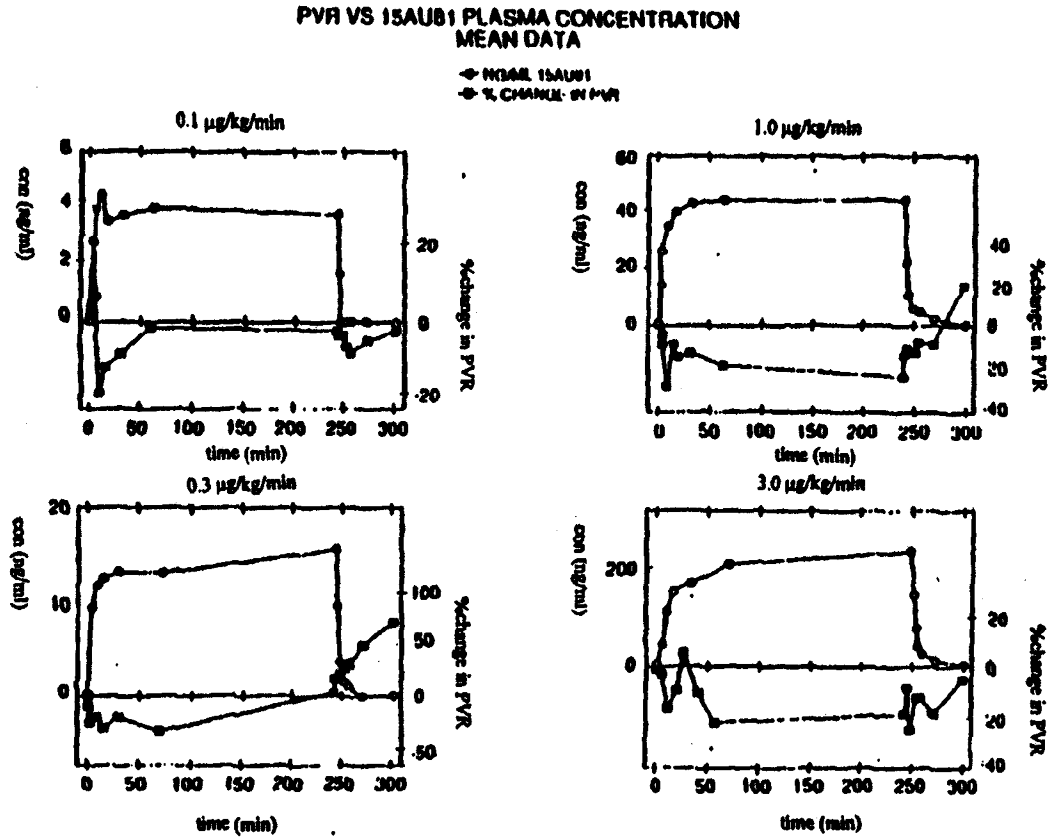


Figure 7. Graphs of mean plasma concentrations of 15AU81 and mean changes in pulmonary vascular resistance (PVR) vs. time, when 15AU81 was infused at 0.1, 0.3, 1.0, and 3.0 µg/kg/min to anesthetized dogs.

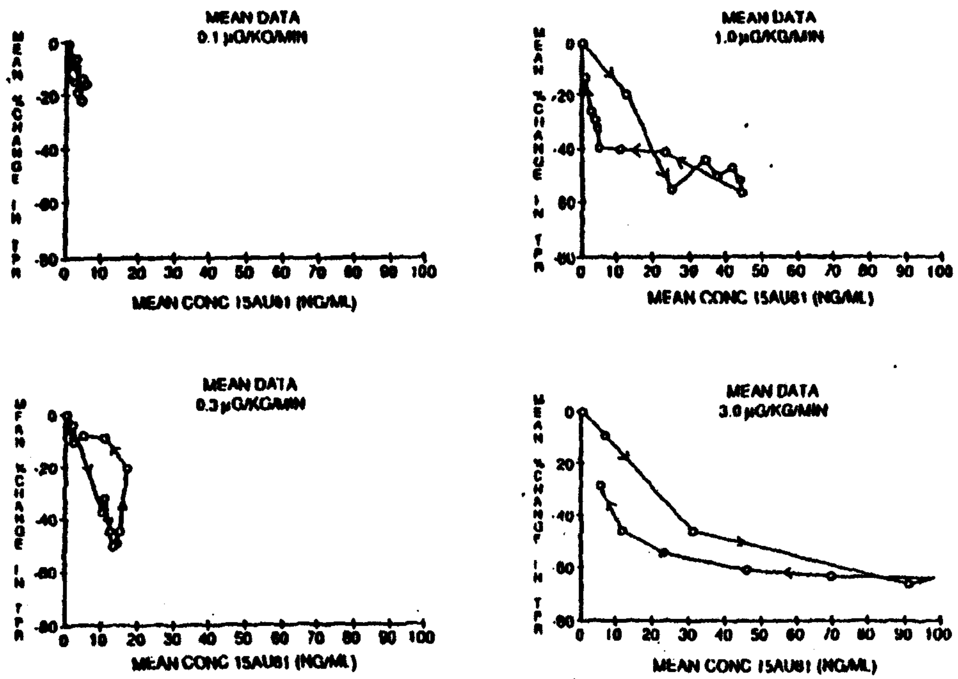


Figure 8. Graphs of mean plasma concentrations of 15AU81 vs mean changes in total peripheral resistance (TPR), when 15AU81 was infused at 0.1, 0.3, 1.0, and 3.0 µg/kg/min to anesthetized dogs. The data points are connected in temporal sequence. The four curves show the concentration-effect plots at each of the four infusion rates, and are equally scaled for ease of comparison. Part of the data from the 3.0 µg/kg/min infusion were not graphed in order to equally scale the x-axis for all four infusions.

Table 11. : **SUMMARY OF PHARMACOKINETIC PARAMETERS OBTAINED IN RATS AND DOGS ADMINISTERED UT-15 BY VARIOUS ROUTES**

Rat	Route	Dose	Label	F	T ½ (hr)	CL**	Cmax**	Tmax (hr)
BPAT 86/18-1	iv	200 µg/kg	[^{3}H]		9-14	274-441	-	-
	po	200 µg/kg	[^{3}H]	45.9	3.6-7.4	-	-	-
BPAT 87/60	po	5 mg/kg	[^{3}H]	30.7	4-24	276-440	-	-
Dog								
BPAT 87/3-1	po	20 µg/kg	[^{3}H]	116-130	12.1	25.8	5.2-18	0.5-3
	iv		[^{3}H]	-	3.3	24.6	-	-
TBZZ/90/0064	iv-inf	0.1-3 µg/kg/min	-	-	0.33	-	-	-
TBZZ/90/0068	po	200 µg/kg	-	62	-	-	96.6	0.75
	iv	20 µg/kg	-	-	0.05-0.33	-	-	-
	it	20 µg/kg	-	46	~0.2***	-	11.9	0.12
	it+Exo	20 µg/kg	-	88	~0.3***	-	12.5	0.12
TBZZ/90/0049	iv-inf	0.05 µg/kg/min	-	-	-	-	LOQ*	-
	iv-inf	0.1 µg/kg/min	-	-	-	-	≤ 2.9*	-
	iv-inf	0.2 µg/kg/min	-	-	-	-	2-6*	-

It must be emphasized that the half-life data obtained using total radioactivity does not specifically represent the true half-life of UT-15.

*Data were taken at the end of the infusion. The assay LOQ is [redacted]

**Clearance values are in mL/hr/kg and Cmax values are in ng/mL or ngEq/mL

***Data were estimated graphically.

Tissue Distribution Studies

Two tissue distribution studies were performed in rats, one using oral tritium-labeled drug and the other with the carbon-labeled drug given subcutaneously.

In the first study, the tissue distribution of total tritium was determined in a qualitative whole body autoradiographic study in male and female albino (Sprague-Dawley; 6/sex) and male pigmented (PVG/C; n=4) rats given a single oral dose of 200 µg [^{3}H] UT-15/kg. The rats were killed at 1, 4 and 24 hr post-dose and were processed for whole body autoradiography. The above time points represent the tissue distribution of radioactivity at about times of peak concentrations of tritium in plasma, at one half-life of total tritium after peak concentrations, and at 24 hours after dosing. There were no major differences in the distribution of radioactivity between males and females or between albino and pigmented rats. At one hour after dosing, the majority of radioactivity was seen in the stomach and small intestine. Of the other organs, the liver (bile canaliculi) and kidney (cortico-medullary junction) contained the most radioactivity. At 4 hr after dosing, the distribution of radioactivity was similar to that seen at 1hr. At 24 hr post-dose, of the small amount of radioactivity still remaining, the majority was in the large intestine, with not much radioactivity seen in any other organs including the liver and kidney. The results indicated that the majority of the drug after an oral dose remained in the GI tract and was excreted in the feces, and the portion of the drug material that was absorbed into the system was excreted in the bile and urine.

In the second study, the tissue distribution of radioactivity was examined in 6 male Sprague-Dawley and 4 male Long Evans rats following administration of a single 6-hr sc infusion of [^{14}C]UT-15 at a targeted dose of 450 ng/kg/min. Sprague-Dawley rats were sacrificed (one animal/time point) at the end of infusion (EOI), and at 1, 2, 4, 8 and 48 hr from the EOI. Long Evans rats were sacrificed (one animal/time point) at the EOI, and at 12, 24 and 72 hr from the EOI. Blood and tissues were collected from all animals and were analyzed for total radioactivity.

The concentrations of radioactivity found in blood, plasma and various tissues are presented in Table 12. The maximum concentrations (C_{max}) of radioactivity in blood and plasma were 12.7 and 23.6 ng equivalents [^{14}C]UT-15/g, respectively. Distribution of radioactivity was rapid, with most tissues reaching maximum concentrations within 2 hr from the EOI. The tissues with the highest C_{max} were liver, small intestine, nonpigmented skin, kidneys, pigmented skin and large intestine, with concentrations of 624, 409, 139 (EOI average), 110, 93.9 and 92.3 ng equivalents [^{14}C]UT-15/g, respectively. The tissues with the lowest C_{max} values were the brain and fat with 1.12 and 1.38 ng equivalents/g, respectively. Blood and tissue concentrations declined with time, and by 72 hr post-dose radioactivity was not detected in 10 of the 29 tissues examined.

In the above study, it was determined that following sc infusion of [^{14}C] labeled drug, the radioactivity was eliminated from blood and plasma with half-lives of 90.2 and 53.7 hr, respectively. The half-life values in tissues ranged from 1.66 hr (thyroid) to 478 hr (fat). The half-life values for pigmented and nonpigmented skin were mostly the same, suggesting that [^{14}C]UT-15-derived radioactivity does not bind to melanin.

It is noted that all half-life data represents total radioactivity including parent and metabolites.

Table 12.

Concentration of Radioactivity in Blood, Plasma, and Tissues at Specified Times After Administration of a Single 6-Hour Subcutaneous Infusion of [^{14}C]UT-15 (162 μg/kg) to Male Rats

	ng Equivalents [^{14}C]UT-15/g				
	Animal Number/Time Point (Strain)				
Matrix	C35583/ EOI (LE)	C35577/ EOI (SD)	C35588/ 1 Hour (SD)	C35579/ 2 Hours (SD)	C35580/ 4 Hours (SD)[b]
Abdominal aorta					
Adrenal glands					
Bladder (urinary)					
Blood					
Bone (femur)					
Bone marrow (femur)					
Brain					
Cellular fraction					
Eyes (both)					
Fat (reproductive)					
Heart					
Kidneys					
Large intestine (including cecum)					
Liver					
Lungs					
Mesenteric lymph nodes					
Muscle (thigh)					
Pancreas					
Plasma					
Prostate					
Salivary glands					
Skin (nonpigmented)					
Skin (pigmented[a])					
Small intestine					
Spleen					
Stomach					
Testes					
Thymus					
Thyroid					

a Long Evans rats only.
b *The 4-hour animal was an apparent misdose; therefore, the data was excluded from all calculations.*

LE Long Evans.
SD Sprague Dawley

EOI End of infusion.
ND Not detected.
NS No sample.

Table 12 (contd.)

Concentration of Radioactivity in Blood, Plasma, and Tissues at Specified Times After Administration of a Single 6-Hour Subcutaneous Infusion of [^{14}C]UT-15 (162 μg/kg) to Male Rats

Matrix	ng Equivalents [^{14}C]UT-15/g Animal Number/Time Point (Strain) C35581/ 8 Hours (SD)	C35584/ 12 Hours (LE)	C35585/ 24 Hours (LE)	C35582/ 48 Hours (SD)	C35586/ 72 Hours (LE)
Abdominal aorta					
Adrenal glands					
Bladder (urinary)					
Blood					
Bone (femur)					
Bone marrow (femur)					
Brain					
Cellular fraction					
Eyes (both)					
Fat (reproductive)					
Heart					
Kidneys					
Large intestine (including cecum)					
Liver					
Lungs					
Mesenteric lymph nodes					
Muscle (thigh)					
Pancreas					
Plasma					
Prostate					
Salivary glands					
Skin (nonpigmented)					
Skin (pigmented[a])					
Small intestine					
Spleen					
Stomach					
Testes					
Thymus					
Thyroid					

a Long Evans only.

LE Long Evans.
SD Sprague Dawley.

EOI End of infusion.
ND Not detected.
NS No sample.

Metabolism Studies

Rat Liver Microsomal Cytochrome P450 Isozyme Induction Potential

Three groups of male Sprague-Dawley rats (5/group) were administered UT-15 (200 ng/kg/min, continuous sc infusion for 7 days), vehicle control (continuous sc infusion for 7 days) or phenobarbital (a positive control, 80 mg/kg ip, once daily for 8 days), respectively. All animals were killed 24 hr after the termination of sc infusion or the last ip injection. Livers were collected and hepatic microsomal fractions were prepared and analyzed for total protein content, total cytochrome P450 content, and isozyme activities of CYP1A, CYP2B and CYP3A. Results indicated that the administration of UT-15 had no effect on the yield of hepatic microsomal protein, total cytochrome P450 content, or isozyme activities. Phenobarbital administration produced statistically significant increases in total cytochome P450 content and isozyme activities of CYP1A, CYP2B and CYP3A.

Human Hepatic Microsomal Cytochrome P450 Isozyme Inhibitory Potential

The inhibitory effects of UT-15 on selected human cytochrome P450 isozymes were examined in human liver microsomal preparations. Assays specific for six human liver microsomal cytochrome P450 isozymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A) were performed at a single substrate concentration over a wide range of UT-15 concentrations (0.1, 1.0, 10, 100 and 1000 ng/ml) with appropriate positive controls, to determine if UT-15 inhibits metabolism of any of the substrates. The concentration of UT-15 that inhibits 50% of the activity (IC_{50}) of each specific isozyme was determined. The IC_{50} value for each isozyme was found to be greater than 1000 ng/ml, indicating that UT-15, at the tested concentration range, did not significantly inhibit the activities of any of the six P450 isozymes studied.

Other Studies

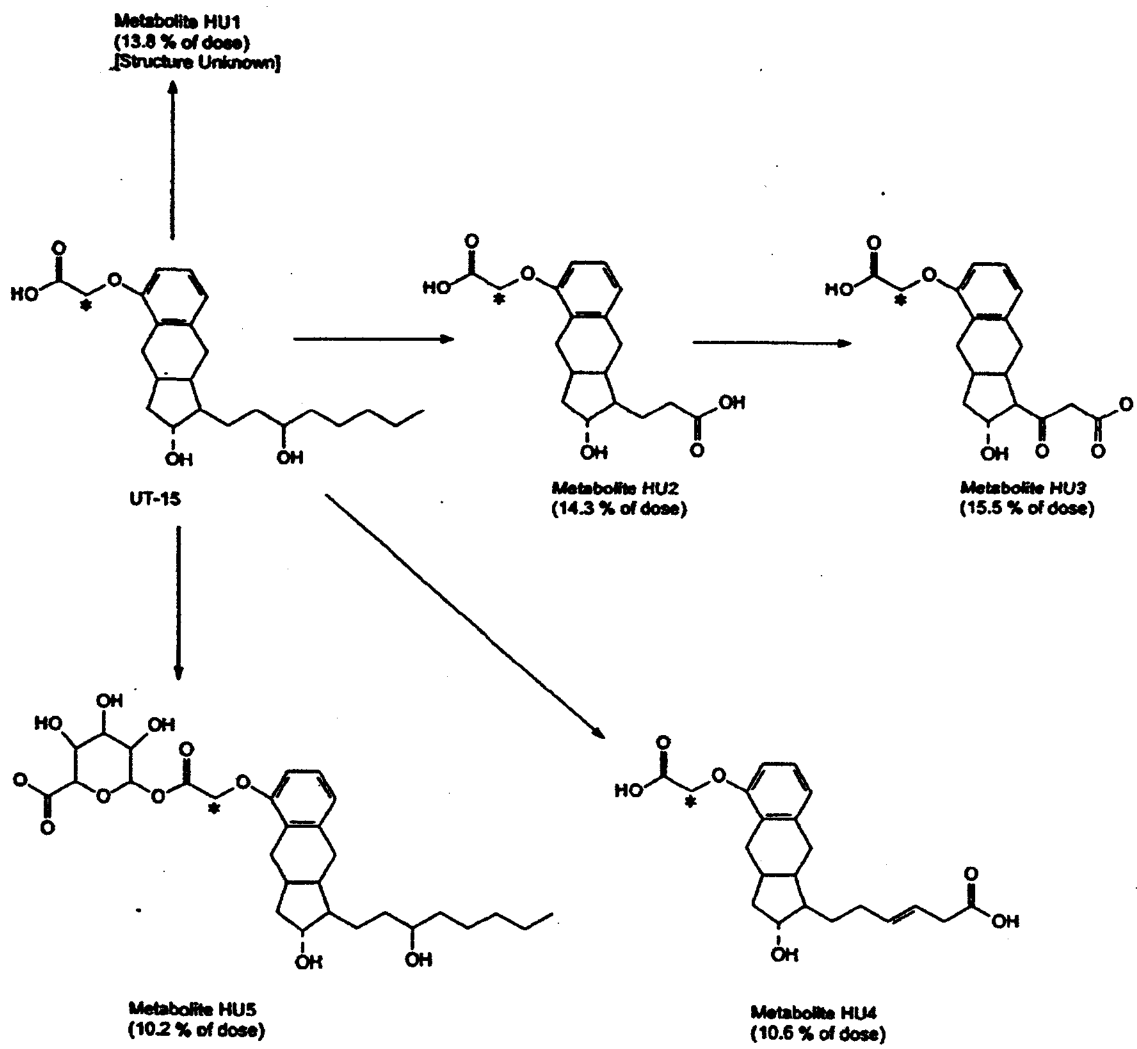
The metabolic profile of UT-15 was examined in bile collected from rats (2/sex/treatment) given single oral or iv administration of 200 µg [^{3}H]UT-15/kg. Analysis of the bile sample showed little or no unchanged UT-15, suggesting extensive metabolism of UT-15 to more polar compounds. Incubation of the bile sample with beta-glucuronidase/aryl sulphatase showed the presence of glucuronide and/or sulfate metabolites.

No metabolic studies were conducted in the dog.

The metabolic profile of UT-15 was investigated in human urine obtained from six healthy male volunteers given sc infusion of [^{14}C]UT-15 at 15 ng/kg/min for 8 hours. [redacted] analysis of the urine samples showed that [redacted] of the administered dose was excreted as unchanged drug. There was no single major metabolite. Five urinary metabolites (HU1, HU2, HU3, HU4 and HU5) accounted for 13.8, 14.3, 15.5, 10.6 and 10.2% of the dose, respectively. HU5 was identified to be UT-15-glucuronide. HU4, HU3 and HU2 were the products of oxidation of the 3-hydroxyloctyl side-chain. The structure of HU1 has not been elucidated. The proposed metabolic pathway is shown in Figure 9.

Figure 9.

Proposed Metabolic Pathway



In a separate study, urine was collected from healthy volunteers (8 males and 7 females) administered the drug by sc infusion at 15 ng/kg/min for 2.5 hours. Over 48 hours, 4.9% of the dose was excreted as unchanged drug and 10.4% of the dose was excreted as UT-15-glucuronide. Sulfate conjugates were not present.

Plasma Protein Binding Studies

The *in vitro* binding of [^{14}C]UT-15 to female human plasma protein (pooled sample from 3 subjects) was assessed by [redacted] at two concentrations, [redacted] The mean protein binding of UT-15 in human plasma was 91% at UT-15 concentrations of both [redacted] suggesting that the plasma protein binding of UT-15 appears to be concentration independent. It was also shown that UT-15 did not significantly affect the *in vitro* protein binding of [^{3}H]digoxin and [^{14}C]warfarin in human plasma.

Protein binding studies were not performed using rat or dog plasma because the ^{14}C labeled UT-15 was unstable even at [redacted]

Excretion Studies

The excretion of UT-15 was studied in male and female Sprague-Dawley rats (3/sex) after administration of a single oral dose of 200 µg [^{3}H]UT-15/kg. A mean total recovery of 97.3% of the administered radioactivity was obtained over the 72 hr post-dosing period (includes cage wash and 1-2% from carcass). The major route of elimination was fecal with a mean total recovery of 82.1%. About 13.2% was eliminated in urine and 0.1% in the expired air. There was no significant difference in the excretion of total [^{3}H] between males and females.

In a separate study, to examine the role of biliary excretion in the elimination of UT-15-related material, bile was collected at hourly intervals (over the 24 hr duration of the study) from rats (2/sex/treatment) with cannulated bile ducts, after a single oral or iv dose of 200 µg [^{3}H] UT-15/kg. Urine and feces were also collected for the same period. Mean total recoveries of 79.9 and 96% of the administered [^{3}H] were obtained after oral and iv dosing, respectively. There was no significant gender difference in the excretion of total [^{3}H]. Bile was the major route of excretion, irrespective of the route of administration, accounting for 67.1% of the oral dose and 91.1% of the iv dose.

An excretion and pharmacokinetic study was conducted in rats using [^{14}C] labeled drug administered by the subcutaneous route. Eighteen male Sprague-Dawley rats were divided into two groups; Group 1 consisting of 15 bile-duct intact rats and Group 2 with 3 bile duct cannulated rats. Animals of both groups were given single sc infusion of [^{14}C]UT-15 at 450 ng/kg/min for 6 hours. Blood was collected from Group 1 animals at various time points during the infusion and continuing through 120 hr postdose for radio-activity level determinations. Urine, feces and bile were collected from Group 2 rats through 120 hours post infusion.

Twenty-four hours after infusion termination, the concentrations of radioactivity in the cellular fraction of blood were much lower than that found in the plasma. The maximum concentration (C_{max}) of radioactivity in plasma occurred at 0.25 hours after the end of infusion and averaged 45.9 ng equivalents/g. The maximum concentration of radioactivity in the cellular fraction of blood occurred at the end of the infusion and averaged 9.35 ng equivalents/g. The concentrations in the plasma and cellular fraction declined slowly, with plasma showing a terminal half-life of 24.6 hours. Radioactivity was still detectable in plasma and cellular fractions at 120 hours after the end of infusion.

About 88 and 99% of the administered dose was excreted within 24 hours following the termination of infusion in bile duct intact and bile duct cannulated animals, respectively. Feces was the major route of elimination in bile duct intact animals, with mean values of 82.2% excreted in feces and 14.1% in urine. In bile duct cannulated animals, bile was the major route of excretion. The radioactivity in bile, urine and feces accounted for 89.5, 10.5 and 1.05% of the administered dose, respectively. The overall mean recoveries were 96.5% in bile duct intact and 101% in bile duct cannulated animals.

The excretion of total radioactivity was studied in male and female dogs (2/sex) administered single iv or oral doses of 20 µg/kg [^{3}H]UT-15/kg. Total recovery of administered radioactivity averaged 97.0% after the iv dose and 83.8% after the oral dose. There was no gender difference in the excretion of drug-related material. After iv administration, about 70% of the administered dose was recovered in the feces and 26% in the urine. After oral dosing, about 65% of the dose was recovered in the feces and 18% in the urine.

In human volunteers (6 males), who were given a single 8-hour sc infusion of [^{14}C]UT-15 (15 ng/ kg/min), urinary excretion was the main route of elimination of [^{14}C]UT-15-derived radioactivity (about 79% of the administered dose). Fecal elimination accounted only for about 13% of the administered dose. This is in contrast with the main route of excretion [fecal (bile)] seen in rats and dogs.

The excretion data obtained from various species are presented in Table 13.

APPEARS THIS WAY
ON ORIGINAL

TABLE 13 : BALANCE/EXCRETION OF TOTAL RADIOACTIVITY FROM RATS, DOGS, AND MAN ADMINISTERED UT-15 BY VARIOUS ROUTES

Rat	Route	Dose	Label	Recovered Label (%)	Feces (%)	Urine (%)	Air (%)	Bile (%)
BPAT 86/17	po	200 µg/kg	[^{3}H]	97.3	82.1	13.2	0.1	-
Pilot Study	sc	200 µg/kg	[^{14}C]	87.0	67.1	10.2	1.2	-
Covance 7049-104	sc	23 µCi/kg	[^{14}C]	96.3	82.2	14.1	-	- -
Dog								
BPAT 87/3-1	iv	20 µg/kg	[^{3}H]	97.0	70.4	25.7	-	-
	po	20 µg/kg	[^{3}H]	83.8	64.9	18.1	-	-
Man								
Protocol P01:10	sc	1.1 µg/kg	[^{14}C]	92.2	13.4	78.6	-	-

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

SUMMARY OF GENERAL TOXICITY STUDIES

Acute and 14-Day Subcutaneous Toxicity Studies in Rats and Dogs

These studies with UT-15 (Lot No. UA) were conducted at [redacted] in accordance with GLP regulations.

Rats: An acute dose rangefinding study ([redacted] study number 585F-104-420-97; August 1 to 14, 1997) was conducted to determine the maximum dose of UT-15 that did not produce adverse clinical signs in Sprague-Dawley rats when administered as a continuous subcutaneous infusion for a period of 3 hours. One female rat was given an initial target dose of 400 ng/kg/min using a [redacted] infusion pump. A second rat received a target dose of 500 ng/kg/min. The rats were observed continuously during the infusion and hourly for 6 hours after dosing.

There were no treatment-related clinical signs in the first rat. At approximately 490 ng/kg/min (achieved dose), slight ataxia was noted in the second rat (16 min after the initiation of dosing). The severity of ataxia had decreased by about 1.5 hours post initiation of infusion, and at 2.5 hours, ataxia was no longer seen.

No clinical signs were noted during the 6-hour post infusion observation period.

These animals were not necropsied.

A 14-day continuous subcutaneous infusion study ([redacted] study number 585B-102-430-97; September 12 to October 08, 1997) was performed in Sprague-Dawley rats (5-8/sex/group), using implanted [redacted] osmotic pumps, at target doses of 50, 150 or 450 ng/kg/min. Reversible significant decreases in red blood counts, hemoglobin and hematocrit values were observed in the low and mid dose groups (both sexes), relative to controls, with an inverse relationship between dose and the hematological effects. Increases in reticulocyte counts, suggestive of a regenerative hematological response, were also noted in the above groups. There were no significant hematological findings in the high dose group of either sex.

There were no treatment-related findings for body weight, food consumption, clinical chemistry, organ weight and gross or microscopic pathology parameters.

Dogs: An acute dose rangefinding study ([redacted] study number 585G-503-420-97; September 3 to 10, 1997) was conducted in Beagle dogs to determine the maximum dose of UT-15 that did not produce adverse clinical signs when given by continuous subcutaneous infusion for a period of 3 hours. One male dog was given an initial target dose of 300 ng/kg/min using a [redacted] infusion pump. Subsequent doses were increased by about 100 ng/kg/min increments when no clinical signs were noted. The dog was observed continuously during the infusion and hourly for 6 hours after dosing.

No clinical signs were detected at infusion rates of approximately 304, 406 or 507 ng/kg/min. At 608 ng/kg/min, vomiting was observed 1 hour after the initiation of infusion. The dog appeared normal one hour after dosing. No other abnormalities were observed either during or after the infusion period.

No necropsy was performed.

In the above 3 hour infusion study, the maximum dose that did not result in adverse clinical signs was about 500 ng/kg/min.

A 14-day continuous subcutaneous infusion study (— . study number 585C-501-430-97; September 18 to October 10, 1997) was performed in Beagle dogs (2-3/sex/group), using a —— infusion pump, at target doses of 50, 200 or 400-600 ng/kg/min. Due to deaths in high dose male animals, the initial high dose of 600 ng/kg/min was lowered twice (for both sexes), by 100 ng/kg/min decrements, to a final high dose of 400 ng/kg/min. One high dose male dog was found dead on study day 3 and the high dose was lowered to 500 ng/kg/min. A second high dose male was found dead on day 6 and the dose was subsequently lowered to 400 ng/kg/min. On day 11, the last high dose male dog (with a rectal prolapse) was sacrificed in extremis. Gross necropsy examination of these dogs revealed intestinal intussusception in two of three dogs, with one having a rectal prolapse. No gross lesions were seen in the third dog. Histopathologically, moderate intussusception with hemorrhage, inflammatory cellular infiltrate, necrosis of the everted ileum and prolapse and edema of rectum were seen. Similar gastrointestinal findings were not observed in high dose females.

The clinical signs observed in high dose animals included hypoactivity, emesis and loose stool. Edema with or without skin lesions at the injection sites was seen in treated and control dogs.

There were no treatment-related hematology, clinical chemistry, EKG and organ weight findings in this study.

The no observed adverse effect level in this study was 200 ng/kg/min.

APPEARS THIS WAY
ON ORIGINAL

Twenty-six Week Subcutaneous Continuous Infusion Toxicity Study in Rats Followed by a 4-Week Recovery Period

Testing Facility: [redacted]

Study Number: 1224-98 ([redacted] Lab.'s No.)

Study Dates: Initiation of Treatment - November 9, 1998
Terminal Sacrifice – May 10 to June 7, 1999

GLP Compliance: The study was conducted in compliance with GLP regulations.

Animals: Sprague-Dawley rats [redacted], 15 rats/sex/group, housed individually in stainless steel wire mesh-bottom cages and fed *ad libitum* with [redacted] Rodent Diet #8728C, were about 8 weeks old (males weighed 272 to 346 g and females 169 to 237 g) at the beginning of the study. An additional five rats/sex/group were assigned to the vehicle control and high dose groups for the recovery phase of the study, while an additional 4 rats/sex/group were assigned to all drug-treated groups for toxicokinetic evaluations.

Dose Levels and Mode of Administration: The target dose levels and the concentrations of dosing solutions are given below.

Treatment Groups	*Target Dose Levels*[1]		*Concentration*
	ng/kg/min	*Mg/kg/day*	*mg/ml*
1. Saline control[2]	0	0	0
2. Vehicle control[3]	0	0	0
3. Low dose	50	0.072	0.06
4 .Mid dose	150	0.216	0.18
5. High dose	450	0.648	0.54

[1] The test and control articles were administered at a constant rate of 0.05 ml/kg/hr to achieve target dose levels. [2] Saline control animals received 0.9% sodium chloride for injection USP. [3] Vehicle control animals received the vehicle containing sodium citrate, citric acid, sodium chloride and meta-cresol dissolved in Water for Injection USP (pH 6.8 – 7.4).

UT-15 (Lot No. UT15RP-98I001) solutions were prepared by dilution of appropriate volumes of UT-15 stock solution (10 mg/ml) with the vehicle to achieve the desired concentrations. The dosing solutions were filtered through a [redacted] filter to assure sterility. The test and vehicle articles were prepared once every 2 weeks, and were stored refrigerated.

Analyses of test article concentrations at three different time points during the study showed that the concentrations of the dosing solutions were about 92 to 98% of the nominal values.

It is stated that "the dose levels were selected by the sponsor based on available data."

Animals were dosed by continuous subcutaneous infusion, 24 hours/day. The animals were anesthetized using CO_2/O_2 gas ([redacted] . the infusion site on the dorsal side was surgically prepared, and a catheter was inserted subcutaneously and secured in place. The catheter was connected to medical grade tubing that was passed to the outside of the cage, and then connected to the reservoir containing the test article solution. The infusion rate was controlled using an infusion pump.

The infusion site was changed to another dorsal site every 7 days for the first 12 weeks of the study, and then less frequently (every 14 days) for the remainder of the study, to reduce the stress associated with repeated anesthesia.

[Note: A topical antiseptic (0.05% chlorhexidine gluconate) was applied to the surgical sites of all animals throughout the treatment period. Animals (both control and treated) exhibiting skin lesions (wound, dermatitis, ulceration, edema and erosion) were treated with topical applications of 1% iodine and/or 4% chlorhexidine gluconate. Additionally, three mid dose animals exhibiting ocular lesions were treated with topical applications of an ocular lubricant ([redacted]).]

<u>Observations/Measurements</u>: Animals were observed daily for clinical signs and mortality. A detailed clinical examination was performed one week prior to initiation of dosing and once weekly thereafter, with particular attention paid to the surgical sites. Body weights and food consumption were recorded for all animals one week prior to initiation of treatment, and weekly during the treatment and recovery periods. Indirect ophthalmoscopy and slit lamp examinations were performed on all animals during the pretreatment period and again for the main and recovery phase animals during weeks 13 and 26 of the treatment period. Hematological [RBC, WBC (total and differential), platelet and reticulocyte counts, hemoglobin, hematocrit, MCV, MCH, MCHC, prothrombin time, activated partial thromboplastin time, and blood cell morphology], and serum chemistry (urea nitrogen, creatinine, glucose, cholesterol, triglycerides, total protein, albumin, globulin, ALT, AST, alkaline phosphatase, bilirubin, calcium, sodium, potassium, phosphorus and chloride) evaluations were performed on all main study animals during week 13 and also prior to necropsy at week 26, and on all recovery animals at the end of the recovery period. Urinalysis was also conducted at the above time intervals.

About 1 ml of blood was collected from the orbital sinus or the jugular vein of the toxicokinetic phase animals at pretreatment and on Days 1, 7, 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, and 182 for toxicokinetic evaluations. Blood samples were collected 3 hours post start of infusion on Day 1, and at the same hour on each of the remaining occasions. After the last blood sample collection, these animals were discarded without further examination.

All main phase animals were sacrificed after 26 weeks of treatment, and all recovery phase animals were kept for an additional 4 weeks (without treatment) before sacrifice.

Complete necropsies were performed on all animals, including those that died or were sacrificed in moribund condition. Adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, prostate, spleen, testes, thymus, thyroid (with parathyroid) and uterus were weighed. Representative sections of adrenals, aorta (thoracic), brain, cecum, cervix, colon, epididymides, esophagus, eyes, femoral bone, heart, infusion sites, kidneys, lacrimal glands, liver, lungs, lymph nodes (mesenteric and mandibular), mammary gland, optic nerves, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin and subcutis, small intestine, spinal cord (cervical), spleen, sternum and marrow, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus and vagina, and all abnormal tissues were collected and fixed in neutral buffered 10% formalin (except epididymides, eyes, optic nerves and testes which were fixed in Zenker's fluid).

Three femoral bone marrow smears were prepared from each animal, but were not evaluated.

Tissues for microscopic examination were processed and stained with hematoxylin and eosin/phloxine. All tissues collected from vehicle control, high dose (including recovery phase animals), and dead or moribund-sacrificed animals were examined histologically. Gross lesions from all animals and infusion site tissues from all recovery phase animals were also examined histologically.

Data were statistically analyzed for homogeneity of variance using Levene Median and for normality using Kolmorogov-Smirnov tests. For the main phase animals, homogeneous data were analyzed using the analysis of variance and the significance was tested by Dunnett's t test. Heterogeneous data were analyzed using Kruskal-Wallis test and the significance was tested using Dunn's test. The significance of inter-group differences for the recovery phase animals was assessed using the student t test.

Results: The most frequently observed clinical signs in animals of all dose groups, including controls, included the presence of lumps, swellings and/or thickening of the skin at or around the infusion site. The incidence and frequency of occurrence of infusion site findings were higher in all drug-treated groups compared to control groups, and also, the incidence of lesions at the site of infusion was greater in the high dose animals than in lower dose group animals.

Dose-related increased incidences of redness of the nose, pinnae, paws and/or tail were seen in drug treated animals, the males being more affected than females.

A total of 29 animals (24 from the main and recovery phase groups and 5 from the toxicokinetics groups) died or were sacrificed in extremis during the study (Table 14). The treatment day of an individual animal's death or moribund sacrifice and the cause of death for the main and recovery phase animals are presented in Table 15. (Four of 5 deaths from the toxicokinetics groups were anesthesia related.)

Table 14.

Number of Animals Found Dead or Preterminally Euthanized in Each Dose Group

Treatment Groups	Target Dose Levels		Number of Animals					
			Main Phase		Recovery Phase		Toxicokinetic Phase	
	mg/kg/day	ng/kg/min.	Males	Females	Males	Females	Males	Females
1. Saline Control	0	0	3	0	N/A	N/A	N/A	N/A
2. Vehicle Control	0	0	4	0	1	0	N/A	N/A
3. Low Dose	0.072	50	4	1	N/A	N/A	1	0
4. Mid Dose	0.216	150	2	2	N/A	N/A	1	0
5. High Dose	0.648	450	4	2	1	0	1	2

N/A= Not Applicable

Table 22.

Preterminal Deaths and Sacrifices of Main and Recovery Phase Animals

Animal ID	Dose Level (ng/kg/min.)	Sex	Circumstance	Day	Cause of Death/Euthanasia
1004B	0	M	Found dead	84	Renal failure
1108C	0	M	Moribund sacrifice	31	Obstruction of urinary tract
1015E	0	M	Found dead	105	Obstruction of urinary tract
2002A	0	M	Moribund sacrifice	165	Obstruction of urinary tract
2009C	0	M	Moribund sacrifice	153	Granulocytic leukemia
2011D	0	M	Moribund sacrifice	135	Obstruction of urinary tract
2013E	0	M	Moribund sacrifice	81	Obstruction of urinary tract
2019F	0	M	Moribund sacrifice	153	Obstruction of urinary tract
3006B	50	M	Found dead	78	Anesthesia-related death
3008C	50	M	Found dead	162	Anesthesia-related death
3010D	50	M	Found dead	134	Anesthesia-related death
3014E	50	M	Found dead	144	Anesthesia-related death
3511D	50	F	Found dead	92	Anesthesia-related death
4008C	150	M	Found dead	19	Obstruction of urinary tract
4012D	150	M	Found dead	124	Obstruction of urinary tract
4505B	150	F	Found dead	162	Anesthesia-related death
4513E	150	F	Moribund sacrifice	127	Renal failure
5003A	450	M	Moribund sacrifice	136	Obstruction of urinary tract
5004B	450	M	Moribund sacrifice	165	Obstruction of urinary tract
5012D	450	M	Moribund sacrifice	150	Obstruction of urinary tract
5013E	450	M	Found dead	32	Obstruction of urinary tract
5017F	450	M	Found dead	53	Obstruction of urinary tract
5503A	450	F	Found dead	120	Anesthesia-related death
5504B	450	F	Found dead	176	Anesthesia-related death

M: Males F: Females

Of the 24 deaths (19 males and 5 females) that occurred in the main and recovery phase animals, 13 (all males; both control and treated) were attributed to urinary tract obstruction, 8 (4 males and 4 females; treated groups) to anesthesia for catheter implantation procedures, 2 (1 control male and 1 mid dose female) to renal failure and 1 (vehicle control male) to granulocytic leukemia. For males that died due to urinary tract obstruction, the necropsy findings included presence of large volume of urine in the bladder or rupture of the urinary bladder, and thickening and discoloration of the urinary bladder. Histologically, dilated renal cortical tubules with proteinaceous material in the lumina, minimal tubular degeneration with increased number of mitoses, and occasional tubular casts as well as dilatation and/or hemorrhage of the urinary bladder were observed. These findings were accompanied by inflammation of the urinary bladder, prostate or seminal vesicles, prostatic hemorrhage and edema of surrounding soft tissues. Since the number of deaths or moribund sacrifices in drug-treated and control groups were comparable, and since the pathology findings were generally similar, the deaths were not considered to be treatment related. [Although anesthesia related deaths were seen only in drug treated rats, no dose relationship for this finding was noted (low dose – 5, mid dose – 1, & high dose – 2)]

No treatment-associated effects on body weight and food consumption were noted.

There were no drug-related ocular findings.

Increases in mean white blood cell counts (16–40%), compared to saline or vehicle ontrol animals, were noted in the high dose animals of both sexes during treatment weeks 13 and 26. At the end of the recovery period, WBC counts were comparable in the vehicle control and high dose groups.

Although not statistically significant, dose-dependent increases in total bilirubin (8-45%) were noted in treated groups (both sexes) during week 26. At the end of the recovery period, the mean total bilirubin level in the high dose male group was significantly higher than that of the vehicle control value. No significant difference in total bilirubin levels was noted between high dose female and vehicle control groups.

Urinalyses revealed no significant findings.

Dose-dependent increases (statistically significant only at the high dose level) in the absolute (males 9 to 23% and females 3 to 16%) and relative (males 6 to 17% and females 8 to 25%) spleen weights were seen in drug treated groups compared to vehicle control groups. Significantly increased absolute heart weights (17%) in males and relative heart weights (11 to 13%) in both sexes were observed at the high dose level. At the end of the recovery period, no significant organ weight differences were seen between high dose and vehicle control groups.

Macroscopically, dark areas, thickening, and nodules or masses were seen at the infusion sites of animals from all groups; however, the incidence and/or severity of the above lesions were higher in drug-treated groups than in controls.

Histopathological findings related to treatment were seen at the infusion site and included edema, hemorrhage, cellulitis, abscess and fibrosis. The incidence and/or severity of these lesions were higher in drug-treated groups than in controls. After the 4-week recovery period, no acute cellular inflammatory changes were seen. Generally, minimal lesions (edema, hemorrhage, fibrosis or pigmentation) were noted in a few animals from either of the two examined groups (high dose and vehicle controls).

There were no other significant or otherwise remarkable histopathological findings.

The mean plasma UT-15 concentration versus study day for each of the three targeted doses is shown in Figure 10. Individual animal data showed wide intra-animal and inter-animal variations in plasma UT-15 concentrations. Steady-state plasma concentrations (C_{ss}) were achieved in most animals by 3 hours on day 1 and in all animals by day 7. The mean steady-state concentrations and the clearance rates are presented below. Male rats were found to have higher plasma UT-15 levels and lower plasma clearance values than female rats. Linear regression analysis of steady-state plasma concentration versus targeted dose data yielded a straight line with coefficient of determination values (r^2) of 0.91 and 0.89 for males and females, respectively, indicating that in both sexes, the steady-state concentration values increased in a dose-related manner.

STEADY-STATE CONCENTRATIONS AND CLEARANCE RATES IN RATS ADMINISTERED UT-15 IN A 26-WEEK TOXICOKINETIC STUDY

Parameter	Dose (ng/kg/min)	Female Rats N = 4	Male Rats N = 3**	Mean
C_{ss} (ng/mL)	50	2.6	3.4	3.0
	150	6.5	10.1	8.3
	450	14.7*	24.7	19.7
CL/F (ml/kg/min)	50	20.8	15.2	18.0
	150	27.8	18.4	23.1
	450	61.2*	23.1	42.2

* n = 2; ** One animal in this group died.

Figure 10.

Plots of Mean UT−15 Concentration (±SEM) versus Study Day by Target Dose

Gender=Female

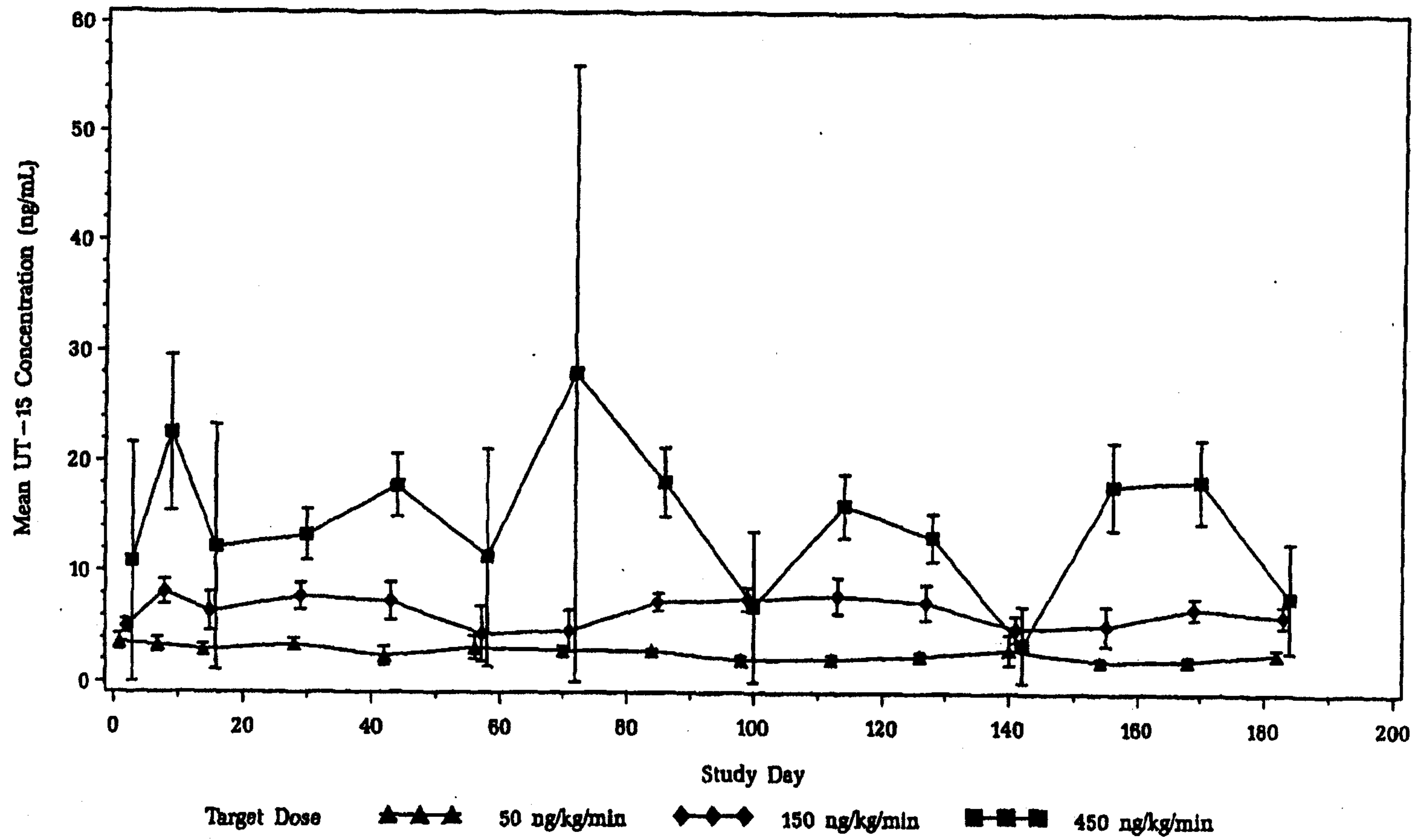


Figure 10 (contd.)

Plots of Mean UT-15 Concentration (±SEM) versus Study Day by Target Dose

Gender = Male