Sponsor's Conclusion: This study demonstrates the specific binding of Technetium Tc99m P829 to somatostatin receptors expressed in human lung and dermal microvascular endothelial cell membranes.

Reviewer's Comment: Agree. However, this study provides little support for the proposed indication of technetium Tc 99m P829. It does support the diffuse distribution of somatostatin receptors throughout the body.

R4.67. Effect of P829 and Somatostatin on Arginine-Induced Glucagon Release in Male Rats. Laboratory, Diatide, Inc., Report Dated January 21, 1998. Lot # 9609B02 (Final Formulation). Report in Volume 1.11 p 272.

Design: Somatostatin and somatostatin analogues are known to inhibit the release of gastrointestinal and pancreatic hormones. This study was designed to evaluate the effect of peptide P829 on arginine-stimulated glucagon release in the rat. P829 peptide was administered as a bolus intravenous dose at 1, 3, 15 or 50 μg/kg to define a dose-response phenomenon. Anesthetized male Sprague-Dawley rats, fasted overnight, were dosed with P829 intravenously at ten minutes prior to the initiation of the arginine infusion. Following the arginine infusion, blood samples were obtained for 1 hour for plasma glucagon determinations.

Results: Area under the plasma glucagon curve following arginine stimulation was determined for each P829 pretreatment. The AUC value for the untreated control was 7857 pg/ml•min. The AUC values for the treatment groups were 7874 pg/ml•min, 7805 pg/ml•min, 6007 pg/ml•min and 4741 pg/ml•min for the 1, 3, 15 and 50 μg peptide/kg dose groups, respectively.

Sponsor's Conclusion: Peptide P829 administered at 1 or 3 μg peptide/kg has little effect on glucagon secretion in the rat as a result of arginine stimulation. Notable suppression was demonstrated at 15 and 50 μg peptide/kg.

Reviewer's Comment: Agree. The inhibitory influence of somatostatin and somatostatin analogues on arginine stimulated glucagon secretion was used as a physiologic parameter to measure the pharmacologic activity of P829 peptide. The glucagon response in pretreated male Sprague-Dawley rats at P829 peptide doses of 1 or 3 µg P829/kg resulted in no adverse effect on the post-prandial hormonal response. At 15 and 50 µg P829/kg glucagon release was greatly reduced. The NOEL for this study was 3-X MHD (by body weight).

25a. Pharmacology Summary

Somatostatin is a multifunctional tetradecapeptide with the peptide sequence illustrated below. A disulfide linkage between the cysteine residues provides a cyclic conformation to the peptide.

Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys
Amino acid sequence of somatostatin.

Somatostatin is found in the hypothalamus and somatostatinergic neurons of the central nervous system, and is also distributed in the gut, endocrine and exocrine glands of the pancreatic islet cells and salivary glands. Somatostatin modulates growth hormone release and inhibits the release of thyrotropin. Additional physiologic functions include inhibition of the release of glucagon, insulin and gastrin. In the gastrointestinal tract, somatostatin decreases intestinal absorption, blood flow and mucosal cell proliferation. The site of action of somatostatin is the cell membrane receptor. Five high-affinity somatostatin receptors have been designated SSTR 1, 2, 3, 4 and 5. These receptors are integral membrane glycoproteins coupled to G-protein. SSTR 2, 3 and 5 belong to one class, with SSTR 1 and 4 belonging to a separate subclass. Somatostatin and similar compounds bind to tumors that are well-differentiated or have neuroendocrine features.

The cyclic hexapeptide domain of the P829 peptide component of Technetium Tc 99m P829 Injection contains the pharmacophore tyrosine-D-tryptophan-lysine-valine which binds to the somatostatin receptor of tumor cells. The potential to detect and localize receptor-expressing tumors in the lung is dependent upon the peptide-receptor interaction. The in vitro binding properties of Technetium Tc 99m P829 Injection were observed in rodent tumor cell membranes, human tumor cell lines, primary human tumors and COS7 cells transfected with the human somatostatin receptor subtypes. The in vivo tumor uptake was demonstrated in several rodent tumor models. In AR42J rat pancreatic tumor membrane preparations, the oxorhenium-complex of P829 (ReO-P829) exhibited a binding affinity 30 times greater than the parent P829 peptide. In the CA20948 rat pancreatic tumor model a high receptor-specific and saturable in vivo tumor uptake was shown. The affinity of P829 and the oxorhenium complex, ReO-P829, for the somatostatin receptor in tumors was demonstrated by the inhibition of ^{125}I-somatostatin binding to receptors on tumor membranes. The affinity of ReO-P829 in the human NCI-H69 and rat pancreatic AR42J tumor membranes was greater than the affinity of P829 alone.

Twenty immortalized cell lines representing various classes of cancer, including breast cancer, lymphomas, SCLC, NSCLC, melanoma, pancreatic cancer, and colon cancer, were assayed for Technetium Tc 99m P829 binding. Technetium Tc 99m P829 bound with high affinity to somatostatin receptors on membranes of cell lines derived from human breast cancer, SCLC, NSCLC, Burkitt's lymphoma, Hodgkin's lymphoma, colon and MIA PaCa2 pancreatic cancer.

In separate studies, Technetium Tc 99m P829 bound with high affinity and capacity to primary and immortal tumor cell lines and to transfected COS7 cells expressing SSTR2, 3 and 5. The

binding of Tc 99m P829 to SSTR3 was displaceable by unlabeled P829/ReO-P829, somatostatin 14, and vasoactive intestinal peptide (VIP). The binding of Tc 99m P829 to SSTR2 and 5 was displaced by P829/ReO-P829 and Tyr^3-octreotide but not VIP.

In describing the SSTR affinity of Technetium Tc 99m P829 with radiochemical purity of 85% and 94%, the dissociation constants of Tc 99m P829 for the SSTR in the AR42J rat pancreatic tumor membrane preparations were essentially equivalent at 12.3 nM and 10.3 nM, respectively. The in vivo biodistribution in the mouse AR42J rat pancreatic tumor xenograft model of the 85% and 94% radiochemical purity preparations of Tc 99m P829 was equivalent as evidenced in the tumor uptake and concentration of radiolabel in the blood and muscle. Similar findings were noted in the mouse NCI-H69 human lung-tumor model.

The two isomers of Technetium Tc 99m P829 present in the reconstituted Kit for the Preparation of Technetium Tc 99m P829 are designated syn and anti. These isomers are present in a ratio of syn/anti = 0.074. Both the syn and the anti isomers bind to SSTR expressed in the AR42J tumor membranes with high affinity. The anti isomer is primary and had a greater receptor affinity.

The biodistribution study in the mouse with the AR42J rat pancreatic tumor xenograft model showed that both isomers were efficacious for tumor targeting by specifically binding to the SSTR upregulated in the AR42J rat pancreatic tumor. The anti isomer had a higher tumor uptake than the syn isomer. Excess octreotide blocked tumor uptake of the isomers and the Technetium Tc 99m P829 Injection, thus indicating that targeting was specific for the SSTR. In biodistribution studies in the Lewis rat CA20948 pancreatic tumor model, the isomeric forms had equivalent tumor uptake. It was noted that the Tc 99m P829 exhibited a greater tumor uptake than either of the isomeric forms. Tumor uptake was also blocked by pretreatment with octreotide.

The specific binding of Technetium Tc 99m P829 to somatostatin receptors expressed in human tumors surgically excised post-imaging was demonstrated in lung tumors which were positive upon Technetium Tc 99m P829 imaging. Specific binding of Technetium Tc 99m P829 to SSTR expressed by human microvascular endothelial cells was shown in a single point determination of Tc 99m P829 binding in the absence and presence of somatostatin-14. Based on Kd of 1, the Bmax values were 195 and 176 fmol/mg protein, for human lung and dermal microvascular endothelial cells, respectively. The binding accounts for the generalized biodistribution of Technetium Tc 99m P829 to the soft tissue in man.

The inhibitory influence of somatostatin and somatostatin analogues on arginine stimulated glucagon secretion was used as a physiologic parameter to measure the pharmacologic activity of P829 peptide. The glucagon response in P829-pretreated male Sprague-Dawley rats at P829 peptide doses of 1 or 3 μg P829/kg resulted in no adverse effect on the post-prandial hormonal response. At 15 and 50 μg P829/kg suppression of glucagon release was greatly reduced.

26. SAFETY PHARMACOLOGY: None Conducted.

27. PHARMACOKINETIC/TOXICOKINETIC STUDIES:

R4.38. Distribution of Technetium Tc 99m P829 Between Human Blood Components In Vitro. Diatide, Inc. Report in Volume 1.15, pp 100-115.

Design: This study was designed to observe the distribution of Tc 99m P829 (0.1, 1.0 and 10 nM) among the components of citrated human blood in vitro as determined by radiometric analysis.

Results: Technetium Tc 99m P829 (%) Associated with Blood Components

Citrated Human Blood Fraction	Percent Technetium Tc 99m P829 Recovered (N = 3)
Plasma	97.27 ± 0.55 %
(Protein Bound)	(1.89 ±0.08 %)
Mononuclear White Blood Cells	0.18 ± 0.05 %
Platelets	0.61 ± 0.20 %
Polymorphonuclear Neutrophils	0.03 ± 0.06 %
Red Cell	0.42 ± 0.05 %

Reviewer's Comment: The majority of the recovered Technetium Tc 99m P829 in the citrated human blood was recovered in the cell-free plasma after density gradient separation. There was less than 2% Tc 99m P829 associated with plasma proteins at the three concentrations tested (0.1, 1.0 and 10 nM).

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R4.27. Pharmacokinetics and Biodistribution of Technetium Tc 99m P829 Injection in Male and Female Rats. Diatide, Inc. Report in Volume 1.15, pp 116-140.

Design: This study was designed to assess the pharmacokinetic properties and biodistribution of Technetium Tc 99m P829 in male and female Sprague-Dawley rats, administered intravenously 100 - 200 μCi Tc 99m/animal in a peptide dose of 1 μg P829 peptide/kg . Blood samples were obtained from 1 through 240 minutes after dosing and tissues and excreta collected for measurement of radioactivity.

	N			Dose			
Group	m	f	Treatment	μCi/animal	μg peptide/kg	Sampling time (min)	Sacrifice time (min)
1	6	-	Tc 99m P829 Injection	100 - 200	1	1, 35, 10, 15, 30, 60, 90, 120, 180, 240	240
2	-	7	Tc 99m P829 Injection	100 - 200	1	1, 35, 10, 15, 30, 60, 90, 120, 180, 240	240

Results:

Pharmacokinetic Parameters for Technetium Tc-99m P829 Injection in Male and Female Rats (Mean ± Standard Deviation)

Parameter	Both Sexes	Males	Females
C_{b0} (%ID/g)	1.99 ± 0.55	1.62 ± 0.58	2.30 ± 0.35
V_C (mL/kg)	303 ± 92	325 ± 108	285 ± 76
$t_{1/2}\alpha$ (min)	1.92 ± 0.44	1.78 ± 0.44	2.04 ± 0.47
$t_{1/2}\beta$ (min)	33.0 ± 7.9	34.1 ± 6.4	32.0 ± 9.9
AUCt $_{0\rightarrow 240min}$ (%ID• min/g)	35.8 ± 8.3	29.9 ± 5.5	40.9 ± 7.6
$V_{D\ ss}$ (mL/kg)	732 ± 183	792 ± 190	680 ± 172
K_{elim_half} (min)	12.3 ± 3.5	12.9 ± 4.2	11.7 ± 3.2
Cl_{tot} (mL/min/kg)	6.2 ± 1.2	6.5 ± 1.4	5.9 ± 0.9
%ID e_GI tract (%ID)	2.4 ± 0.6	2.3 ± 0.8	2.6 ± 0.5
%ID e_urine (%ID)	25.8 ± 8.7	19.8 ± 7.6	30.9 ± 7.1

Average Organ Distribution of Radioactivity in Male and Female Rats at Four Hours After Intravenous Injection of Techentium Tc 99m P829 Injection (Mean ± SD)

Tissue	Both Sexes	Males	Females
Blood, %ID/g	0.017 ± 0.007	0.016 ± 0.008	0.017 ± 0.005
Liver, %ID/g	1.27 ± 0.007	0.94 ± 0.34	1.54 ± 0.22
Kidneys, %ID	38.4 ± 7	44.7 ± 3.5	33.0 ± 4.3
GI Tract, %ID	2.43 ± 0.64	2.28 ± 0.77	2.56 ± 0.52
Liver, %ID	10.33 ± 4.4	9.3 ± 3.4	11.2 ± 5.5
Spleen, %ID	0.37 ± 0.13	0.34 ± 0.14	0.39 ± 0.13
Urine$_{0\rightarrow 240\ min.}$, %ID	25.8 ± 8.7	19.8 ± 7.6	30.9 ± 7.1
Carcass, %ID	7.4 ± 3.3	8.9 ± 4.1	6.2 ± 2.0
Avg. Overall Recovery, %ID	84.7 ± 8.6	85.3 ± 7.7	84.2 ± 9.8

Rats injected with Technetium Tc 99m P829 Injection cleared radioactivity rapidly from the blood biexponentially. The average distribution and elimination half-lives (males and females combined) were 1.9 and 33 minutes, respectively. The apparent volume of the central compartment (V_C) of 303 mL/kg, and the apparent volume of distribution at steady state (V_{Dss}) of 732 mL/kg for both sexes, indicate that Tc 99m P829 distributes to volumes greater than the interstitial space and total body water, respectively. By four hours post-injection, the majority of radioactivity (64 %ID) was associated with the mechanisms of renal excretion, e.g. urine (25.8 %ID) and the kidneys (38.4 %ID). An additional 10 %ID was associated with the liver and 2-3 %ID with the gastrointestinal tract.

R4.74. Pharmacokinetics and Biodistribution of Technetium Tc 99m P829 Injection in Sprague-Dawley Rats with Experimental Renal Dysfunction. Diatide, Inc. Report in Volume 1.15, pp 141-168.

Design: This study was designed to assess the changes in the pharmacokinetic and biodistribution profiles of Technetium Tc 99m P829 in Sprague-Dawley rats with experimental renal dysfunction (ERD; bilateral ligation of the renal artery, renal vein and ureter) compared to sham-operated (S-O) controls.

Group		N			Dose	
		m	f	Treatment	μCi/animal	μg peptide/kg
1	S-O	2	-	Tc 99m P829 Injection	100 - 200	1
2	ERD	3	-	Tc 99m P829 Injection	100 - 200	1
3	S-O	-	3	Tc 99m P829 Injection	100 - 200	1
4	ERD	-	3	Tc 99m P829 Injection	100 - 200	1

Whole blood samples were obtained at 1, 3, 5, 10, 15, 30, 60, 90, 120, 180, and 240 minutes after administration of Technetium Tc 99m P829 Injection, and counted to determine the percent of injected dose per gram (%ID/g). Whole blood time-radioactivity curves were generated and data fitted to an open two-compartment model.

Results:

Pharmacokinetic Parameters for Technetium Tc-99m P829 Injection in Male and Female Rats (Mean ± Standard Deviation)

Parameter	Males		Females	
	Sham-Operated (n=2)	ERD (n=3)	Sham-Operated (n=3)	ERD (n=3)
C_{b0} (%ID/g)	2.47 ± 0.28	2.62 ± 0.70	2.44 ± 0.28	2.26 ± 0.14
V_C (mL/kg)	144 ± 2.1	150 ± 35	139.2 ± 5.7	158.0 ± 7.9
$t_{1/2}\alpha$ (min)	1.58 ± 0.2	1.93 ± 0.0.6	1.64 ± 0.2	2.16 ± 0.2
$t_{1/2}\beta$ (min)	35.3 ± 12.7	216 ± 57	31.7 ± 5.2	161.1 ± 10.5
$AUCt_{0\rightarrow240m}$ (%ID•min/g)	42.3 ± 7.3	124.6 ± 25	40.4 ± 2.7	111.5 ± 4.6
V_{Dss} (mL/kg)	434 ± 62	499 ± 47	412.8 ± 63.1	466.8 ± 35.4
K_{elim_half} (min)	11.2 ± 3.6	61.4 ± 7.2	10.5 ± 2.1	53.0 ± 3.6
Cl_{tot} (mL/min/kg)	3.02 ± 1.1	0.52 ± 0.22	3.10 ± 0.53	0.68 ± 0.07
%ID e_GI tract (%ID)	1.7 ± 0.2	5.1 ± 1.1	2.8 ± 0.8	5.8 ± 0.5
%ID e_urine (%ID)	11.1 ± 1.7	0	19.5 ± 6.2	0

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Average Organ/Tissue Distribution of Tc 99m P829 Radioactivity in Male and Female Rats Combined at Four Hours (Mean ± Standard Deviation)

Organ / Tissue %ID and %ID/g	Sham-operated (n = 5)	ERD (n=6)
Kidneys, %ID	40.6 ± 11.8	3.7 ± 5.6
Liver, %ID	18.1 ± 8.9	30.7 ± 10.1
Spleen, %ID	0.43 ± 0.11	0.73 ± 0.13
GI Tract, %ID	2.06 ± 1.18	5.72 ± 0.72
Urine $_{0\rightarrow 240min}$, %ID	15.7 ± 6.9	0 ± 0
Carcass, %ID	6.6 ± 1.6	43.6 ± 2.1
Liver, %ID/g	1.456 ± 0.17	2.864 ± 0.36
Blood, %ID/g	0.023 ± 0.009	0.365 ± 0.08
Muscle, %ID/g	0.030 ± 0.041	0.090 ± 0.017
Avg. Overall Recovery, %ID	85.9 ± 9.4	94.7 ± 7.7

Under conditions of experimental renal dysfunction both male and female rats exhibited significantly reduced blood clearance of Tc 99m P829. No secondary elimination path was found to compensate.

R4.77 Biodistribution and Pharmacokinetics of Technetium Tc 99m P829 Injection in the New Zealand White Rabbit. Diatide, Inc. Report in Volume 1.15, pp 198-219.

Design: This study was designed to assess the pharmacokinetics and biodistribution of Technetium Tc 99m P829 in 6 New Zealand White rabbits. Sex distribution was not recorded. Rabbits were administered Tc 99m P829 Injection by marginal ear vein at a dose level of 1 μg peptide/kg. Blood samples were obtained from 1 through 240 minutes after dosing. At sacrifice, selected tissues and excreta were collected for measurement of radioactivity.

			Dose		Blood	
Group	N	Treatment	mCi/animal	μg peptide/kg	Sampling time (min)	Sacrifice time
1	3	Tc 99m P829	2.5	1	1, 3, 5, 10, 15, 30, 60, 90, 120, 180, 240	90 minutes
2	3	Tc 99m P829	2.5	1	1, 3, 5, 10, 15, 30, 60, 90, 120, 180, 240	24 hours

Results:

Pharmacokinetic Parameters for Technetium Tc-99m P829 Injection in the New Zealand white rabbit.

Pharmacokinetic Parameter	Mean Value ± Standard Deviation
C_{b0} (%ID/g)	0.313 ± 0.05
V_C (mL/kg)	139.5 ± 9.3
V_{Dss} (mL/kg)	368.1 ± 24.0
MRT (min)	81.3 ± 8.1
$t_{1/2\alpha}$ (min)	2.4 ± 0.8
$t_{1/2\beta}$ (min)	60.7 ± 6.0
$AUCt_{0\rightarrow 240\ min}$ (%ID•min/gm)	9.6 ± 2.2
$AUC\ t_{0\rightarrow\infty}$ (%ID•min/gm)	10.8 ± 2.6
$K_{elim\ half}$ (min)	21.6 ± 4.3

Biodistribution of Technetium Tc 99m P829 Injection in the New Zealand White Rabbit at 90 Minutes and 24 Hours After Injection.

Parameter	90 Minutes (n=3)	24 Hours (n=3)
Percent injected dose/g tissue (% ID/g)		
Liver	0.07 ± 0.003	0.04 ± 0.01
Lungs	0.15 ± 0.05	1.30 ± 1.02
Bile	0.43 ± 0.11	0.30 ± 0.04
Muscle	0.0068 ± 0.002	0.0006 + 0.0001
Pancreas	0.038 ± 0.017	0.010 ± 0.003
Bone Marrow	0.019 ± 0.002	0.0122 ± 0.0024
Articular bone (femur head)	0.081 ± 0.02	0.035 ± 0.017
Spine:		
lumbar bone - disc and perichondral	0.082	0.058
lumbar bone - mid vertebrae	0.025	0.010
proximal aorta	0.18 ± 0.11	0.038 ± 0.014
Percent injected dose (% ID)		
Kidneys	32.4 ± 1.2	26.8 ± 2.6
Liver	7.1 ± 0.3	2.83 ± 0.18
Lungs	1.30 ± 1.02	0.46 ± 0.09
Spleen	0.13 ± 0.09	0.1 ± 0.02
Urine	9.4 ± 4.6	22.9 ± 4.7
Gastrointestinal tract	12.1 ± 2.2	0.63 ± 0.38
TOTAL	62.4±1.57	53.7±1.33

Rabbits injected with Tc 99m P829 Injection cleared radioactivity rapidly from the blood ($t_{½\alpha}$ = 2.5 minutes). The volume of the central compartment (V_C) was approximately 140 mL/kg and the volume of distribution at steady state (V_{Dss}), approximately 368 mL/kg. Tc 99m P829 was distributed primarily to the kidneys (and urine), liver and gastrointestinal tract at 90 minutes after administration. A notable feature of Tc 99m P829 distribution in the eviscerated rabbit carcass was the distribution to the spine and articulation tissues. Bone marrow, while demonstrating some uptake, was five-fold less on a %ID/g basis relative to articulation tissues. Elimination occurred principally in the urine as 23% ID was measured by 24 hours.

R4.71. Pharmacokinetics, Distribution, Metabolism and Elimination of Technetium Tc 99m P829 Injection in the Rhesus Monkey. Diatide, Inc. Report in Volume 1.15, pp 220-235.

Design: This study was designed to assess the pharmacokinetics, biodistribution, metabolism and excretion of Technetium Tc 99m P829 in one rhesus monkey.

	N		Dose		Blood
Group	f	Treatment	mCi/animal	μg peptide/kg	Sampling time (min)
1	1	Tc 99m P829	1.74	1	1, 3, 5, 10, 15, 20, 30, 40, 60, 120, 180, 240

Pharmacokinetic Parameters for Technetium Tc-99m P829 Injection in the Rhesus monkey

Parameter	Mean Value
C_{b0} (%ID/g)	0.199
V_C, Vol (mL/kg)	100.5
V_{Dss}, Vol (mL/kg)	329.4
AUC $t_{0\rightarrow\infty}$ (%ID•min/kg)	5.10
AUC $t_{0\rightarrow 240\ min}$ (%ID•min/kg)	5.63
$t_{1/2\,\alpha}$ (min)	1.6
$t_{1/2\,\beta}$ (min)	67.4
K_{elim_half} (min)	19.3
Cl_{tot} (mL/min/kg)	1.03

Biodistribution of Technetium Tc-99m P829 Injection in the Rhesus monkey by Region-of-Interest Analysis

Organ/Tissue	Percent Injected Dose
Kidneys	40
Liver	10
Urine	32
TOTAL RECOVERY	82%

Urinalysis indicated that the excreted radioactivity was 95.5% associated with peptide and 4.5% as free Tc 99m. Tc 99m P829 was rapidly distributed to tissues ($t_{½\alpha}$ = 1.6 minutes) following intraveous injection followed by a prolonged elimination half-life ($t_{½\beta}$ = 67 minutes). Tc 99m P829 was distributed predominately to the kidneys (40 % ID) and the liver (10 % ID) at 4 hours. Also, at 4 hours 32% of the injected dose was found in the urine. There was no fecal output during the 4 hours of the trial. Total recovered radioactivity was 82%.

R4.69. Pharmacokinetics and Biodistribution of [^{3}H-Tyr]P829 in Male Sprague-Dawley Rats. Diatide, Inc., Report in Volume 1.15, pp 245-376.

Design: This study was designed to assess the pharmacokinetic properties and biodistribution of [^{3}H-Tyr]P829 in male Sprague-Dawley rats.

Group	N	Treatment	Dose		Sacrifice Time (Hrs.)	Blood Sampling Time (Min.)
			µCi/Animal	µgpeptide/kg		
1	3	[^{3}H-Tyr]P829	103	18	1	-
2	6	[^{3}H-Tyr]P829	103	18	4	1, 3, 5, 10, 15, 20, 30, 60, 90, 120, 180, 240
3	3	[^{3}H-Tyr]P829	103	18	12	-
4	3	[^{3}H-Tyr]P829	103	18	24	-
5	4	[^{3}H-Tyr]P829	103	18	96	-

Results:

Pharmacokinetic Parameters for [^{3}H-Tyr]P829 in Male Sprague-Dawley Rats

Parameter	Mean ± standard deviation
C_{b0} (% ID/g)	1.62 ± 0.16
V_C (mL/kg)	263.4 ± 18.1
$t_{1/2\alpha}$ (min.)	6.80 ± 2.37
$t_{1/2\beta}$ (min.)	57.9 ± 8.65
$AUCt_{0\ 240\text{-min}}$ (%ID·min/g)	47.1 ± 9.2
V_{Dss} (mL/kg)	592 ± 54
$K_{elim\ half}$ (min.)	20.4 ± 3.9
Cl_{tot} (mL/min/kg)	3.22 ± 0.62

Average Organ Distribution of Radioactivity in Male Sprague-Dawley Rats After Intravenous Administration of [^{3}H-Tyr]P829.

Tissue	4 Hours (% ID)	24 Hours (% ID)
Liver	23.4 ± 3.2	17.7 ± 0.4
Kidneys	35.3 ± 5.0	35.1 ± 1.9
Gastrointestinal Tract	1.64 ± 0.89	0.55 ± 0.06
Spleen	0.38 ± 0.10	0.68 ± 0.17
Carcass		9.35 ± 0.42
Urine		16.8 ± 2.4
Gastrointestinal Contents and Feces	13.8 ± 2.05	15.4 ± 2.0

Intravenously administered [^{3}H-Tyr]P829 was rapidly removed from the plasma ($t_{\frac{1}{2}\alpha}$= 7 min.) followed by a slower decline that provided a β-phase elimination half-life for total radioactivity of approximately 58 minutes. At 1, 4, 12, 24 and 96 hour after dosing, the majority of the recovered radioactivity was found in the kidneys and liver. Most of the radioactivity measured in the urine and feces was recovered in the first 24 hours, each amounting to approximately 20% ID. The presence of radioactivity in the feces and gastrointestinal tract contents indicates hepatobiliary excretion.

R4.26. An Evaluation of the Metabolism of Technetium Tc 99m P829 in the Sprague-Dawley Rat. Diatide, Inc. Report in Volume 1.15, pp 169-197.

Design: This study was designed to assess the metabolic fate of Technetium Tc 99m P829 in the Sprague-Dawley rat, both in vitro and in vivo. The in vitro part of the study examined metabolism following incubation of Tc 99m P829, in rat plasma, bile, urine, and kidney homogenates and slices. The stability of high specific activity Tc 99m P829 at 2 mCi/μg was evaluated in each biological medium by incubation at room temperature and at 37°C for one or three hours. In the in vivo part of the study, Tc 99m P829 at 2 μCi/μg was administered intravenously to adult, male Sprague-Dawley rats at 20 μg peptide/kg. Samples of plasma, bile, urine and kidney homogenates and slices were obtained. All samples were

for Tc 99m P829 and metabolites.

Results:

Plasma Samples:

In vitro: Rat plasma with Tc 99m P829 revealed limited metabolism to a more-lipophilic species designated as C when incubated at 37°C or room temperature for 3 hours with the protease inhibitor aprotinin. Marked metabolism was observed in plasma incubated for 3 hours at 37 °C without aprotinin. The more lipophilic isomer of the parent Tc 99m P829, the anti isomer, was reduced in percent area from a control value of 92.1% to 8 % area. The area percentage was unchanged following incubation in plasma. The new, more-lipophilic species approximated 82 % of the area. No new, more were evident.

In vivo: Plasma samples were collected to 120 minutes. The radioactivity at 120 minutes was insufficient for analysis. No evidence of metabolism was found in the 15 or 60 minute plasma samples.

Urine Samples:

In vitro: Urine with Tc 99m P829 and incubated for 3 hours at the two temperatures revealed no metabolic change.

In vivo: Urine flow in bladder-cannulated rats was approximately 2.0 mL/kg/hr. Urine contained 15.8 %ID in 6 hours. Urine samples taken over four 15-minute intervals to 1 hour revealed progressively higher amounts of parent Tc 99m P829 radioactivity. Free-Tc 99m and a family of more-hydrophilic species were evident. Intact Tc 99m P829 accounted for less than 10% of the total area before 30 minutes, and 71% to 60 minutes.

Bile Samples:

In vitro: Incubation of Tc 99m P829 in bile did not result in the generation of metabolites.

In vivo: Little radioactivity was detected in bile collected from rats with experimental renal dysfunction. The 0-90 and 90-180 minute collections of bile, revealed only 0.2 %ID over each interval. This percentage of injected dose did not provide sufficient radioactivity for analysis.

Kidney Homogenates and Slices:

In vitro: Addition of Tc 99m P829 to kidney homogenates resulted in significant generation of a more-lipophilic species, designated as [redacted]C. The anti isomer of Tc 9m P829 was apparently more susceptible to metabolism than the syn isomer. In vitro incubation of Tc 99m P829 at 37°C for 1 hour in the presence of the protease inhibitor aprotinin, did not generate the more lipophilic [redacted]C product. Incubation of 200 μCi/100 ng P829 with slices of fresh, untreated rat kidney generated a family of more lipophilic species. The anti isomer was more labile than the syn isomer with a decrease in [redacted] area from 89.8 % to 60.6 %. A family of more-lipophilic species accounted for 26 % peak area. There was no generation of designated [redacted]C.

In vivo. Kidneys removed from rats at 90 minutes following administration of 20 μg peptide/kg were found to contain approximately 30 %ID. Preferential conversion of the anti isomer into the more lipophilic designated [redacted]C accounted for 72 % peak area by 90 minutes after injection.

In conclusion, Tc 99m P829 was cleared rapidly from the blood following intravenous administration to Sprague-Dawley rats at a dose level of 20 μg peptide/kg. Radioactivity was eliminated via the urine (13-20% ID by 6 hours) and minimally via the bile (0.4% ID over 3 hours). The in vitro metabolism of Tc 99m P829 selectively favored the anti isomer, the more lipophilic of the two parent isomers. The anti isomer was slowly metabolized in plasma to a more lipophilic species identified as [redacted]C. The slow metabolism and rapid blood clearance precludes the plasma as the primary site of Tc 99m P829 metabolism. The majority of the metabolism occurred in the kidney. The Tc 99m P829 metabolism in the kidney, in vivo and as an in vitro homogenate, resulted in the formation of predominately [redacted]C, with the selective biotransformation of the anti isomer. In vitro kidney slices generated a family of more lipophilic species, rather than the singular [redacted]C. [redacted]C was not evident in the urine.

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R4.84 Incubation of P829 Peptide in Human Plasma and Rat Plasma. Diatide, Inc., Report in Volume 1.15, pp 236-244.

Design: This study was designed to assess the metabolic behavior of the peptide component of P829 Peptide after incubation in human and rat plasma. Samples were analyzed by reversed phase high performance liquid chromatography. The purity and recovery of P829 Peptide was determined after incubation in human and rat plasma as compared to a saline control. A 100 µL aliquot of a P829 peptide solution was added to 500 µL of rat plasma to yield a peptide concentration of 600 µg/mL. Another 100 µL aliquot was added to 500 µL of human plasma to yield a peptide concentration of 600 µg/mL. As control, a 100 µL aliquot of P829 peptide solution was added to 500 µL of normal saline. All the samples were incubated at 37°C in a water bath. After 1 hour at 37°C, 100 µL aliquots of P829 peptide from rat and human plasma were added to 400 µL of ice-cooled 0.2% TFA in water. The resulting mixture was vortexed for 1 minute followed by centrifugation at 13000 rpm for 3 minutes to deproteinize the sample. The supernatants were analyzed by reversed phase high performance liquid chromatography. Recovery of P829 peptide was calculated by taking the ratio of the area counts in the control, compared to area counts in rat and human plasma samples.

Results: The analysis of P829 peptide in the control sample showed a peak at a retention time of R_t = 21.2 minutes with a total area of 4.0×10^7 AU•sec. Analysis of P829 peptide incubated in human or rat plasma demonstrated a decrease in the total area of the P829 peptide peak with an increase in oxidized products at R_t = 16 minutes and R_t = 38.5 minutes. The area counts of total peptide in human and rat plasma are shown below.

Relative Recovery of Total Peptide from Human and Rat Plasma (1 hour incubation at 37°C).

Sample	Area (x 10^7 AU•sec)			%Recovery	
	Total Peptide	P829 Peptide	P829 (by area)	Total Peptide	P829 Peptide
0.9% Saline (Initial)	4.51	4.50	-	-	-
0.9% Saline (3 hour)	4.01	3.91	98	89%	86%
Human Plasma	2.68	0.08	2.9	67%	2%
Rat Plasma	2.87	0.02	0.7	72%	0.5%

In conclusion, incubation of P829 peptide in human or rat plasma resulted in oxygenation of P829 peptide. The recovery of P829 peptide from the control was 86%, whereas 72% of total peptide was recovered from rat plasm and 67% of total peptide was recovered from human plasma samples. Of the peptide recovered from human and rat plasma, only ≤ 2% was native P829 peptide.

PHARMACOKINETICS SUMMARY

The pharmacokinetic properties of Technetium Tc 99m P829 Injection have been described by an open two-compartment model in the rat, rabbit and rhesus monkey. Despite minor species differences in some parameters, Technetium Tc 99m P829 was rapidly cleared from the blood ($t_{½\mu}$ = 2 minutes). In all three species the volume of the central compartment exceeded the estimated blood volume, and the volume of distribution was in excess of the extra cellular fluid volume. The Technetium Tc 99m P829 was eliminated from the body (K_{elim_half} = 12 – 22 minutes) with a total clearance of 1-6 mL/min/kg. The pharmacokinetic properties were independent of dose as demonstrated by the blood elimination profiles for Technetium Tc 99m P829 Injection administered at 1 and 20 µg/kg in the rat. Experimental renal dysfunction in the rat significantly reduced elimination. The kidneys were the major route of drug elimination, and in renal impairment/dysfunction there was no notable compensation by the hepatobiliary route of elimination in the rat. Technetium Tc 99m P829 was distributed principally to the kidneys, with >30% of the injected dose detected by 4 hours after administration. Distribution to other organs included the liver and gastrointestinal tract. The rabbit demonstrated uptake into the bone, including articulation sites of the shoulder and knee joints and the spine, with bone marrow concentration less than that at the articulation sites. The pharmacokinetic properties and biodistribution of the unlabeled peptide component of Technetium Tc 99m P829 Injection was evaluated in the rat since 85% of a clinical dose of Tc 99m P829 Injection is unlabeled peptide. The peptide component as [^{3}H-Tyr]P829 exhibited pharmacokinetic properties similar to the technetium-labeled P829. The biodistribution of the [^{3}H-Tyr]P829 to the major organs was qualitatively and quantitatively similar to the distribution of Tc 99m P829. A minor difference was the greater amount of the [^{3}H-Tyr]P829 found in the liver at 4 hours compared to the Tc 99m P829.

Pharmacokinetic Parameters for Technetium Tc 99m P829 Injection in the Rat, Rabbit and Monkey

Parameter	Rat (M/F) (n = 13)	Rabbit (n = 3)	Monkey (n = 1)
C_{b0} (%ID/g)	1.99 ± 0.55	0.313 ± 0.05	0.199
V_C (mL/kg)	303 ± 92	139.5 ± 9.3	100.5
$t_{½\alpha}$ (min)	1.92 ± 0.44	2.4 ± 0.8	1.6
$t_{½\beta}$ (min)	33.0 ± 7.9	60.7 ± 6.0	67.4
$AUCt_{0\ 240\ min}$ (% ID•min/g)	35.8 ± 8.3	9.6 ± 2.2	5.63
V_{Dss} (mL/kg)	732 ± 183	368.1 ± 24.0	329.4
$K_{elim\ half}$ (min)	12.3 ± 3.5	21.6 ± 4.3	19.3
Cl_{tot} (mL/min/kg)	6.2 ± 1.2	1.83 ± 0.4	1.03

Pharmacokinetic Parameters for Technetium Tc 99m P829 Injection in Male Sprague-Dawley Rats with Experimental Renal Dysfunction (ERD) (Mean ± SD)

Parameter	Sham-Operated	ERD
C_{b0} (%ID/g)	2.47 ± 0.28	2.62 ± 0.70
V_C (mL/kg)	144 ± 2.1	150 ± 35
$t_{½\alpha}$ (min)	1.58 ± 0.2	1.93 ± 0.06
$t_{½\beta}$ (min)	35.3 ± 12.7	216 ± 57
$AUCt_{0\_240\ min}$ (% ID•min/g)	42.3 ± 7.3	124.6 ± 25
V_{Dss} (mL/kg)	434 ± 62	499 ± 47
K_{elim_half} (min)	11.2 ± 3.6	61.4 ± 7.2
Cl_{tot} (mL/min/kg)	3.02 ± 1.1	0.52 ± 0.22

Biodistribution of Technetium Tc 99m P829 Injection as Percent Injected Dose in the Rat, Rabbit, Monkey

Tissue	Rat 4 Hours	Rabbit 1.5 Hours	Monkey 4 Hours
Kidney	38.4 ± 7	32.4 ± 1.2	40
Liver	10.33 ± 4.4	7.1 ± 0.3	10
G.I. tract	2.43 ± 0.64	12.1 ± 2.2	-
Spleen	0.37 ± 0.13	0.13 ± 0.09	-
Carcass	7.4 ± 3.3	-	-
Urine	25.8 ± 8.7	9.4 ± 4.6	32

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Comparative pharmacokinetic parameters of [^{3}H-Tyr]P829 and Technetium Tc 99m P829 in the male Sprague-Dawley rat

Parameter	Technetium Tc 99m P829	[^{3}H-Tyr]P829
C_{b0} (%ID/g)	1.99 ± 0.55	1.62 ± 0.16
V_C (Ml/kg)	303 ± 92	263.4 ± 18.1
$t_{½\alpha}$ (min)	1.92 ± 0.44	6.80 ± 2.37
$t_{½\beta}$ (min)	33.0 ± 7.9	57.9 ± 8.65
$AUCt_{0\_240\ min}$ (% ID•min/g)	35.8 ± 8.3	47.1 ± 9.2
V_{Dss} (mL/kg)	732 ± 183	592 ± 54
K_{elim_half} (min)	12.3 ± 3.5	20.4 ± 3.9
Cl_{tot} (mL/min/kg)	6.5 ± 2.0	3.22 ± 0.62

Comparative Biodistribution of [^{3}H-Tyr]P829 and Technetium Tc 99m P829 Injection in the Sprague-Dawley Rat as Percent Injected at 4 Hours Post Injection

Tissue	[^{3}H-Tyr]P829	Technetium Tc 99m P829
Kidney	35.3 ± 4.95	38.4 ± 7
Liver	23.4 ± 3.24	10.33 ± 4.4
GI Tract	1.64 ± 0.89	2.43 ± 0.64
Spleen	0.38 ± 0.01	0.37 ± 0.13
Carcass	-	7.4 ± 3.3
Urine	-	25.8 ± 8.7

28. TOXICOLOGY:

Introduction

The toxicology studies used Technetium Tc 99m P829 Injection as test article. It was prepared from decayed generator eluate and the final formulation "Kit for the Preparation of Technetium Tc 99m P829" intended for marketing, unless otherwise indicated.

Single Dose Toxicology

R4.52 Acute Intravenous Toxicity Study (In Mice). Study No. 96G-2354; In-Life, December 4-18, 1996. Report Dated February 20, 1997. Amended Report Dated February 5, 1998. Lot Number 9609B02 (Final Formulation). In Compliance With GLP. Report In Volume 1.12, pp 31-76.

Design: This study was designed to assess the toxicity of Tc 99m P829 Injection, compared to a saline control, after a single dose in young adult Charles River Swiss albino mice. The test material was supplied by the Sponsor to the test facility in 2 separate components; 1) Kit for preparation of Technetium Tc-99m P829, and 2) Decayed Tc-99m generator eluate. Just prior to dosing, the kits were reconstituted with saline and decayed generator eluate to yield concentrations equal to the recommended human dose. After reconstitution, the vials were heated in a boiling water bath for 10 minutes, then allowed to cool at room temperature for 15 minutes prior to dosing. Three groups of 15 male and 15 female mice weighing 19-35 g, received a single injection via the tail vein at dose levels of 0, 300 or 1000 µg peptide/kg, at a volume of 20 ml/kg. Animals were observed daily for signs of toxicity and were sacrificed at 2 days (5/sex/group) or 14 days (10/sex/group) after dosing. Parameters included body weights, hematology (RBC, Hgb, Hct, platelet count, WBC and differential), clinical chemistry (Na, K, Ca, P, AP, LDH, ALT, AST, BUN, creatinine, glucose, albumin and total protein), organ weights (liver and spleen), gross and histopathology (liver, spleen, lung, heart and kidney in high dose and control groups only).

Results: All animals survived to scheduled termination. All animals in both groups receiving Tc 99m P829 Injection exhibited lethargy and piloerection immediately following injection, which cleared within 24 hours. Severity and duration of these clinical signs were similar for both the 300 and 1000 µg peptide/kg groups. No other clinical signs were reported throughout the remainder of the study. Two low-dose female animals lost weight at 14 days. No significant differences in body weight or body-weight changes were noted between groups. Hematology and clinical chemistry parameters were without treatment-related findings at both the 2 and 14 day periods. Splenic atrophy was reported in one control male at necropsy, with no other gross lesions noted. Liver and spleen weights were similar for all groups. Only incidental histopathological lesions were noted and are considered unrelated to treatment with Tc 99m P829 Injection.

Sponsor's Conclusions: Within the limits of this study, no adverse effects attributable to the single intravenous administration of Tc 99m P829 Injection were noted. Based on these results, the intravenous NOEL of Tc 99m P829 Injection is considered to be 1000 µg peptide/kg for both the male and female albino Swiss mouse.

Reviewer's Comments: Disagree. Clinical signs of lethargy and piloerection were seen in all animals receiving Tc 99m P829 Injection at both 300 and 1000 µg peptide/kg body weight immediately after injection, but these effects cleared within 24 hours. There were no other dose or treatment-related effects seen in this study in either male or female mice.

R4.50 A Single-Dose Two-Day And Two-Week Toxicity Study Of P829 Administered By Intravenous Injection To Rabbits. Study No. A819-911. [redacted] May 27-June 11, 1997. Report Dated December 2, 1997. Lot Number 9609B06 (Final Formulation). In Compliance With GLP. Report In Volume 1.12, pp 78-237.

Design: This study was designed to assess the toxicity of Tc 99m P829 Injection, compared to saline control, in young New Zealand White rabbits. Animals were supplied by [redacted] and designated as Hra:SPF-NZW, and were 4 months of age and weighed 2.3-2.7 kg. The test material was supplied by the Sponsor to the test facility in 2 separate components; 1) Kit for preparation of Technetium Tc-99m P829, and 2) Decayed Tc-99m generator eluate. Just prior to dosing, the kits were reconstituted with decayed generator eluate and saline to yield concentrations equal to the recommended human dose. After reconstitution, the vials were heated in a boiling water bath for 10 minutes, then allowed to cool at room temperature for 15 minutes prior to dosing. Three groups of male and female rabbits (8/sex/group) received a single injection via the marginal ear vein of Tc 99m P829 Injection at dose levels of 0, 200 or 600 µg peptide/kg, and a volume of 12 ml/kg. Animals were observed for signs of toxicity immediately after dosing, and at 1, 2 and 4 hours later. On day 3, three animals per sex per group were sacrificed, with the remaining animals sacrificed 14 days after dosing. Parameters included body weights, hematology (RBC, HGB, HCT, platelets, reticulocytes, WBC and differential), clinical chemistry (Na, K, Cl, Ca, P, BUN, AST, ALT, AP, LDH, GGT, CPK, creatinine, bilirubin, glucose, total protein, albumin, globulin, cholesterol and triglycerides), organ weights (adrenal, brain, liver and spleen), gross and histopathology (44 tissues from control and high-dose only).

Results: All animals survived to scheduled termination. No treatment-related signs of toxicity were observed throughout the study period. No treatment-related effects on body weights and body-weight changes between groups were noted. There were no treatment-related effects on any of the hematology or serum chemistry parameters. Only sporadic, unrelated variations in clinical pathology parameters were observed. There were no gross findings at necropsy and no treatment-related effects on organ weights, except decreased absolute adrenal weight in high-dose females at the 3 day sacrifice. The adrenal/body weight ratio was not affected, so this finding is considered spurious. Histopathological examination of tissues from the control and high-dose animals yielded no noteworthy findings.

Sponsor's Conclusions: Within the limits of this study, no adverse effects attributable to the single intravenous administration of Tc 99m P829 Injection were noted. Based on these results, the intravenous NOEL of Tc 99m P829 Injection is considered to be 600 µg peptide/kg for both the male and female New Zealand white rabbit.

Reviewer's Comment: Agree. There were no effects seen in this study that are considered dose- or treatment-related.

Repeat Dose Toxicology

R4.51 A Ten-Day Repeated Dose Toxicity Study Of P829 In Sprague-Dawley Rats. Study No. 67082. August 5-25, 1994. Report Dated October 18, 1994. Lot Number 940013 . Not In Compliance With GLP. Report In Volume 1.12, pp239-336.

Design: This study was designed to assess the toxicity of Tc 99m P829 (unformulated product; P829 trifluoroacetate reconstituted with Glucoscan®), compared to saline control, after repeated daily intravenous administration for ten days in Charles River Sprague-Dawley rats. Glucoscan® is the kit for preparation of technetium Tc 99m Gluceptate and contains 200 mg Gluceptate sodium and 0.06 mg stannous chloride. Test animals were received in two shipments and from two different Charles River locations, namely, Four groups of male and female rats (10/sex/group) received repeated tail vein injections of Tc 99m P829 at dose levels of 0, 30, 100 or 400 µg peptide/kg/day, and a volume of 1.0 ml/kg. Animals were observed daily for signs of toxicity after each dose. Rats were sacrificed on study day 11, just 24 hours following the last dose on study day 10. Parameters included body weight, hematology, clinical chemistry, organ weights, and gross necropsy. No histopathology was conducted.

Results: All animals survived to scheduled termination. No clinical signs, body weight differences or hematological findings are considered treatment related. Glucose levels in the high-dose male and female rats were decreased compared to the control group. Albumin, albumin/globulin ratio, cholesterol, calcium and phosphorus levels were decreased in high-dose females, with globulin levels increased. There were no differences in terminal body weights between groups. Liver weights (absolute and relative) of the 400 µg peptide/kg/day females and the relative liver weights of the males of the same group were significantly decreased when compared to the control group. No gross observations were noted at necropsy and histopathology was not conducted.

Sponsor's Conclusions: Based on the results of this study, the repeated-dose intravenous NOEL of Tc 99m P829 as P829 trifluoroacetate reconstituted with Glucoscan® is 100 µg peptide/kg/day administered for 10 days for both male and female Sprague-Dawley rats.