Table 253-1:

Mean Distribution of Metabolites after Incubation of [^{3}H]Medetomidine, [^{3}H]Dexmedetomidine and [^{3}H]Levomedetomidine with Human Liver Slices

Metabolite	% Total Metabolites		
	Medetomidine (n=2)	Dexmedetomidine (n=4)	Levomedetomidine (n=2)
G-Dex-1	14.32	22.39	0.67
G-Dex-2	9.32	14.69	2.06
G-levo	44.60	t	71.30
COOH	0.40	1.02	0.27
OH	2.08	10.81	1.14
G-OH	0.61	1.56	0.29
SO_3OH	nd	nd	nd
M-OH	nd	nd	nd
N-Methyl	2.73	10.77	2.52
H-I	1.05	1.54	0.57
Other	24.91	37.22	21.20
Turnover (%)	78.39	54.43	83.02

G-Dex-1 = N-glucuronide of dexmedetomidine
G-Dex-2 = N-glucuronide of dexmedetomidine
G-Levo = N-glucuronide of levomedetomidine
COOH = carboxylic acid metabolite (MPV-1306)
OH = hydroxy metabolite (MPV-1305)
G-OH= O-glucuronide of the hydroxy metabolite
SO_3OH = sulfate conjugate of the hydroxy metabolite
M-OH = mercapturic acid conjugate of the hydroxy metabolite
methyl N-methylated metabolite (MPV-1709)
H-1 = glucuronide of the hydroxylated N-methyl metabolite
Turnover = % Parent drug converted to metabolites
t = trace nd = not detected

Table 253-2:

Variability in the Rate of [^{3}H]Dexmedetomidine Glucuronidation in a Panel of Twenty-One Human Liver Microsomes

Subject ID	pmol/min/mg protein G-Dex-1	G-Dex- 2	G- l/G-2
EGF426*	5.83	4.00	1.46
FAG771	9.67	6.17	1.57
FB1779	38.00	24.42	1.56
EFESS6	65.92	40.17	1.64
FGL852	27.00	17.67	1.53
Flw309	86.50	56.17	1.54
FRX710*	45.58	30.67	1.49
GC4476	49.33	30.00	1.64
GDDS65	11.08	8.42	1.32
GEQS91	75.08	45.33	1.66
GFE06O	93.25	59.67	1.56
GJ5562	12.25	8.67	1.41
HAK163	27.83	18.75	1.48

GLT656	13.67	9.75	1.40
H0DO57*	116.58	73.25	1.59
HF1812	17.42	10.17	1.71
HF0125951	124.17	78.25	1.59
ICF119	36.75	22.83	1.61
IEG710	63.83	40.25	1.59
IENOl6	71.08	43.92	1.62
IE4420	46.25	29.58	1.56
Mean	49.38	31.34	1.55
SD	35.30	22.02	0.10

* received known inducers of UDPGT activity

The underlined values in the above table are demonstration of the extremes in rates of glucurinidation among human subjects 5.83 to 124.17 pmol/min/mg protein, >20 fold difference.

Table 253-3:

Summary of the Kinetic Parameters for the Glucuronidation of Dexmedetomidine by Human Liver Microsomes

glucuronidation Reaction	Parameter*	Subject I.D. EFE	ICF	GDD	Mean +SD
Dex-1	Km (mM)	2.64	3.83	1.67	2.71+ 1.08
	Vmax (pmol/min/mg)	1937	826	268	1010 + 849
	Vmax/Km (mL/min/mg)	0.73	0.22	0.16	0.37 + 0.32
Dex-2	Km(mM)	1.57	2.15	2.61	2.11+0.52
	Vmax (pmol/min/mg)	592	334	332	420 + 150
	Vmax/Km (mL/min/mg)	0.38	0.16	0.13	0.22 +0.14

*units for Vmax and Vmax/Km are expressed relative to mg of microsomal protein

Summary

The metabolism of dexmedetomidine by human liver slices and human liver microsomal preparations indicated that the direct N-glucuronidation was a significant route of metabolism and the metabolic profile is qualitatively similar to *in vivo* results from human plasma and urine. There is a 10 fold difference between subjects in the extent of N-glucuronidation, but the ratio of the metabolites for each nitrogen is relative constant and indicates the preferential glucuronidation at one of the imidazole nitrogen versus the other but apparently both by the same enzyme. [Metabolic pathways: page 72 (rat) and page 100 (human)]

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NDA 21-038

[41]

Study Title: Abbott-85499 Drug Metabolism Report No.36 - Identification of cytochrome P450 isoforms involved in the oxidative metabolism of dexmedetomidine (Abbott-85499) and the effect of dexmedetomidine on cytochrome P450-mediated monooxygenase activities

(#254)

Study No: Report No. R&D/97/757
Vol #45, and page #109:
Conducting laboratory and location: Abbott Laboratories Division 46: Abbott Park, IL
Date of study report: January 1998.

Methods:
Human Liver Microsomes: The human livers of transplant quality were obtained from [redacted]. The liver tissue samples were received within 24 hrs of removal. The fact that CYP2D6 may have a role in dexmedetomidine metabolism was addressed by preparing liver microsomes from an extensive metabolizer (ID1211961, male) and a poor metabolizer (ID415961, male). The tissue was homogenized, centrifuged and the microsomal pellets resuspended in phosphate buffer and stored at -70°C until use in incubations for studies. The microsome incubations with dexmedetomidine was stopped, centrifuged and the supernatants analyzed [redacted]

cDNA-Expressed CYP proteins: Microsomes prepared from B-lymphoblastoid cells containing cDNA-expressed CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYPE1, CYP3A4 and CPY4A11 were obtained from [redacted]. The microsome incubations with dexmedetomidine was stopped, centrifuged and the supernatants analyzed by [redacted]

Selective CYP2A6 Antibody: Inhibition of metabolism of [^{3}H]-dexmedetomidine by a selective CYP2A6 monoclonal antibody to provide more evidence of this isozyme in the *in vitro* metabolism. The antibody was supplied by [redacted]

Drug, lot#, radiolabel, and % purity: Medetomidine, with tritium on the bridge methyl group, was synthesized by [redacted] and the dexmedetomidine (Lot #50498-ST-108; 66 Ci/mmol) was separated at Abbott by chiral chromatography. Unlabeled dexmedetomidine (Lot #031940-002) were added to the labeled compounds only to provide final incubation concentrations greater than 0.05uM. The radiochemical purity was [redacted]

Results:
This study was done to identify which P450 isozymes were involved in dexmedetomidine metabolism and to examine the effects of dexmedetomidine on the isoform-specific cytochrome

P450 mediated reactions.

Oxidative metabolites: The chromatograph of dexmedetomidine metabolites, in the presence of NADPH, typically demonstrated two major metabolic peaks. One of these peaks was identified as MPV-1305, the phenyl-3-hydroxymethyl metabolite of the parent and a late eluting peak was tentatively identified as the methylene bridge hydroxymethyl, identified as the probable H-3 metabolite detected in human plasma *in vivo*.

Inhibition studies: The inhibition of CYP2A6 by 8-methoxypsoralen inhibited the formation of both hydroxymethyl metabolites, MVP-1305 and H-3 by 40 to 60%. However the inhibition of this enzyme by coumarin, inhibited the formation of MVP-1305 by more than 30% but of H-3 by less than 10%. Selective inhibition of CYP3A decrease MVP-1305 formation by 34% in one prep but only 3% in the other microsomal preparation.

Metabolism by cDNA-Expressed Proteins: CYP2D6 and 2A6 exhibited the highest rates of hydroxylation to the MVP-1305 metabolite of dexmedetomidine. The CYP2E1 and CYP2D6 were the most active P450s for formation of H-3 metabolite.

The following tables present the results obtained (V45/p134-5):

Table 254-1 Hydroxylation of Dexmedetomidine in the Presence of Human B-Lymphoblastoid Cell Microsomes Containing cDNA-Expressed CYP Proteins

	Dexmedetomidine Hydroxylation (5 uM)		Dexmedetomidine Hydroxylation (0.05 uM)	
CYP Form	MPV-1305 pmol/hr/pmol	H-3 pmol/hr/pmol	MPV-1305 pmol/hr/pmol	H-3 pmol/hr/pmol
1A2	ND	0.162	0.002	0.004
2B6	ND	0.044	0.004	0.060
2C8	0.204	ND	0.008	ND
2C9-arg	ND	0.742	ND	0.002
2C19	0.295	ND	0.141	ND
2D6-val	4.261	1.7t1	0.664	0.002
2E1	0.467	2.781	0.008	0.049
3A4	0.506	0.955	0.079	0.054
2A6	1.770	0.206	0.058	0.005
4A11	ND	0.306	ND	ND
Control[a]	ND	ND	ND	ND

[a] Microsomes prepared from cells devoid of CYP
ND =No activity detected

Dexmedetomidine inhibition of Cytochrome P450s: The inhibition was most evident on CYP2C9, CYP2D6 and CYP3A, all less than 1uM. The IC_{50} values are presented in Table 254-3 below. The CYP2C9 has a major role in the metabolism of phenytoin, warfarin and NSAIDS. CYP2D6 is involved in metabolism of compounds like codeine and hydroxycodone and the type of dexmedetomidine inhibition appears to be mixed, competitive and non-competitive. It is a polymorphic enzyme and about 5% of caucasians, 2% of asians and africans are deficient in this enzyme. CYP3A, the major form of CYP3 in adults, is about 33% of the total P450 isozymes and it plays a role in sex steroid metabolism, e.g. testosterone. Dexmedetomidine also potently inhibits the N-demethylation of ketamine in human liver microsomes with a Ki of 0.4 uM. The CYP involved in this reaction has not been identified.

Table 254-2 Hydroxylation of Dexmedetomidine in the Presence of Human B-Lymphoblastoid Cell Microsomes Containing cDNA-Expressed CYP Proteins

	Dexmedetomidine Hydroxylation (5 uM)		Dexmedetomidine Hydroxylation (0.05 uM)	
CYP Form	MPV-1305 pmol/hr/pmol	H-3 pmol/hr/pmol	MPV-1305 pmol/hr/pmol	H-3 pmol/hr/pmol
1A2	ND	0.162	0.002	0.004
2B6	ND	0.044	0.004	0.060
2C8	0.204	ND	0.008	ND
2C9-arg	ND	0.742	ND	0.002
2C19	0.295	ND	0.141	ND
2D6-val	4.261	1.7t1	0.664	0.002
2E1	0.467	2.781	0.008	0.049
3A4	0.506	0.955	0.079	0.054
2A6	1.770	0.206	0.058	0.005
4A11	ND	0.306	ND	ND
Control[a]	ND	ND	ND	ND

[a] Microsomes prepared from cells devoid of CYP ND = No activity detected

Summary

The CYP2A6 is apparently the largest contributor to dexmedetomidine hydroxylation in vitro in human microsomes to the metabolites MPV-1305 and H-3, the 3-hydroxymethyl and the bridge carbon hydroxylation, respectively. The CYP inhibition by dexmedetomidine affects several isozymes, most potently CYP2C9 (0.2uM IC_{50}), CYP3A4 (0.65uM IC_{50}), and CYP2D6 (0.4 uM IC_{50}). The sponsor cites clinical data indicating the therapeutic blood level are 0.04 uM or less and claims this precludes any role of dexmedetomidine in metabolism of concomitant drugs. However, the ADME study with labeled dexmedetomidine demonstrated that, upon a single administration, the liver concentrations were nearly 100X the peak plasma concentrations. Since

the metabolising P450s are mainly in the liver, there remains the possibility that dexmedetomidine can interfere with the metabolism of concomitant medications, especially after prolonged administration. The latter situation has not been studied, although it has been proposed in clinical protocols. Studies to address this concern have been proposed by the sponsor, approved by the division and are to be done in the near future.

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[42]

Study Title: Abbott-85499 Drug Metabolism Report No.13 - The in vitro interaction of dexmedetomidine with human liver microsomal cytochrome P450 2D6 (CYP2D6)

(#258)

Study No: Report No. R&D/96/557
Vol #45, page #179:
Conducting laboratory and location: Abbott Laboratories Division 46: Abbott Park, IL
Date of study report: August 1996

Methods: The human livers of transplant quality were obtained [redacted] The liver tissue samples were received within 24 hrs of removal. The tissue was homogenized, centrifuged and the microsomal pellets resuspended in phosphate buffer and stored at -70°C until use in incubations for studies. The microsome incubations with dexmedetomidine was stopped, centrifuged and the supernatants analyzed by [redacted]. [O-methyl ^{14}C] dextromethorphan was also used as a substrate.
cDNA-Expressed CYP proteins: Microsomes prepared from B-lymphoblastoid cells containing cDNA-expressed CYP2D6-Val were obtained from. [redacted] The microsome incubations with dexmedetomidine was stopped, centrifuged and the supernatants analyzed by [redacted]
Drug, lot#, radiolabel, and % purity: [O-methyl ^{14}C] dextromethorphan was synthesized by [redacted] The unlabeled dexmedetomidine, Lot #295260-0-AX [redacted] and the quinidine, Lot #01831KW from [redacted] C]HCHO, 30.1 mCi/mmol, was purchased from [redacted]

Results:
The effects of dexmedetomidine on the Dextromethorphan O-demethylase, CYP2D6, was studied *in vitro*. The Table 258-1 presents the IC_{50} values for dexmedetomidine and quinidine. Although quinidine is about 7-times more potent, dexmedetomidine's K_i was still in range of 1 uM.

Table 258-1:

Inhibition of Dextromethorphan O-Demethylase Activity in the Presence of Dexmedetomidine or Quinidine

Subject ID	IC_{50} (uM)[a] Dexmedetomidine	Quinidine
Human Liver Microsomes		
HF0	1.3	0.18
GC4	1.2	b
FRX	1.5	b
HAK	1.5	0.16
ICF	0.9	0.20
Mean ± SD[c]	1.3 ± 0.25	0.18 ± 0.02
Projected K_i (uM)[d]	0.70	0.09
cDNA-Expressed CYP2D6[e]	2.0	0.15

[a] IC_{50}: Concentration of quinidine or dexmedetomidine required to inhibit activity by 50%. The final concentration of DEXTRO approximated apparent Km.

[b] Not determined. [c] Mean ± standard deviation.

[d] Assuming competitive inhibition. When substrate concentration = Km, $IC_{50}/2 = K_i$.

e Human B-lymphoblastoid cell microsomes containing cDNA-expressed wild type CYP2D6.

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Table 258-2:

Inhibition Constants Characterizing the Inhibition of Human Liver Microsomal Dextromethorphan 0-Demethylase Activity in the Presence of Dexmedetomidine or Quinidine

Parameter[a]	Subject ID ICE	HFO	GC4	Mean ± SD[b]
Dexmedetomidine				
K_i(uM)	0.2	0.5	0.4	0.4 ± 0.2
K_{ies} (uM)	3.2	2.2	1.5	2.3 ± 0.9
Alpha	16	4.4	3.9	8.1 ± 6.8
Quinidine				
K_i (uM)	0.05	0.08	[c]	0.07[d]

[a] K_i, K_{ies} and a (Kies/Ki) were determined using Dixon and Cornish-Bowden plots. Dexmedetomidine behaved as a mixed (competitive/noncompetitive) inhibitor (K_{ies} > Ki; alpha >1) and quinidine exhibited competitive inhibition. [b] Mean + SD of three livers. [c] Not determined.

Summary

The potent inhibition of CYP2D6 by dexmedetomidine was as a reversible mixed (competitive/non-competitive) inhibitor and quinidine, a competitive inhibitor, was 6-7 times more potent. The sponsor again cites that the expected plasma levels of dexmedetomidine (0.04uM) is much lower than the IC_{50} (1.3uM). However, upon single administration of labeled dexmedetomidine to the rat, the liver concentration exceeded the peak plasma level by nearly two orders of magnitude. There has been no study to evaluate possible liver accumulation and yet the sponsor has proposed 5 consecutive days of dexmedetomidine infusion. The CYP inhibition by dexmedetomidine could produce significant changes in metabolism of concomitant medications if there is any liver accumulation of the parent compound or active metabolites.

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NDA 21-038

[43]

Study Title: Abbott-85499 Drug Metabolism Report No.8 - *In vitro* protein binding of [^{3}H]Abbott-85499 (dexmedetomidine) in mouse, rat, dog, monkey, and human plasma (Protocol V96-004) (#242)

Study No: V96-004; Report No. R&D/96/320
Vol #43, page #136:
Conducting laboratory and location: Abbott Laboratories Division 46: Abbott Park, IL
Date of study report: July 1996
Methods: Blood samples were obtained from both sexes of mice (CD, Charles Rivers), rats (Sprague-Dawley, Sasco), beagle dogs (Marshall Research Farms), cynomolgus monkeys (Charles Rivers), and human volunteers. The heparinized blood was centifuged to separate the plasma and appropriate drug solutions were added to the plasma. The solutions were incubated at 37°C in shaking water baths and placed in a centrifuge devise that provided ultrafiltration during centrifugation. The filtrates were extracted with acetonitrile and analyzed chromatographically.
Drug, lot#, radiolabel, and % purity: Medetomidine HCl, with tritium on the bridge methyl group, was synthesized by [redacted] and the dexmedetomidine isomer was separated at Abbott by chiral chromatography. (Lot #53863-MK-085 (80 Ci/mmol; 331 uCi/ug, 1.1 ug/ml). Unlabeled dexmedetomidine (Lot #295260-0-AX), was added to the labeled dexmedetomidine HCl to provide a solution of 10, 5, 2.5 and 1.0 ug salt/ml dose solution. The radiochemical

Results:
The protein binding results are presented in the following table copied from the submission (V43/p146):

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Table 242-1:
Summary of the *In Vitro* Protein Binding of [^{3}H]Abbott-85499 in Mouse, Rat, Dog, Monkey and Human Plasma

Species	Sex	% Protein Binding
Mouse	Mean[1]	94.89
	SD	0.18
Rat	Mean[1]	88.16
	SD	0.31
Dog	Mean[1]	92.58
	SD	0.28
Monkey	Mean[1]	84.59
	SD	0.48
Human	Mean[1]	93.72
	SD	0.40
Saline	4 Day Mean	3.20
	SD	3.10

SD = Standard Deviation
[1] Means are calculated from the male and female results of each concentration tested.

Summary:

The binding in males and females was similar in all species and the percent plasma protein binding ranged from a mean of 84.6% in monkeys to 94.9% in mice and in humans the mean binding was 93.7%.

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[44]

Study: Abbott-85499 Drug Metabolism Report No.20 - *In vitro* binding of [^{3}H]Abbott-85499 (dexmedetomidine) to human serum albumin and α_1- glycoprotein (Protocol V96-0 11)

(#243)

Study No: Report No.R&D/97/338
Vol #43, page #161:
Conducting laboratory and location: Abbott Laboratories Division 46: Abbott Park, IL
Date of study report: June 1997
Methods: Human serum albumin and alpha$_1$-acid glycoproteins were obtained from [redacted] and dissolved in phosphate buffer. The protein binding was determined by an ultrafiltration technique. The solutions were incubated at 37°C in shaking water baths and placed in a centrifuge devise that provided ultrafiltration during centrifugation. The filtrates were extracted with acetonitrile and analyzed chromatographically. The radioassay of the proteins was in a liquid scintillation analyzer.

NDA 21-038

Drug, lot#, radiolabel, and % purity: Medetomidine HCl, with tritium on the bridge methyl group, was synthesized [redacted] and the dexmedetomidine isomer was separated at Abbott by chiral chromatography. (Lot #53863-MK-085; 80 mcCi/mmol) Unlabeled dexmedetomidine, Lot #295260-0-AX, was added to the labeled dexmedetomidine HCl to provide a solution of 8.5, 4.25, 2.125 and 0.85 ug free base/ml. The radiochemical [redacted] %.

Results:

The results are presented in the following tables from the submission (V43/p173-177):

Table 243-2:

In Vitro Binding of [^{3}H]Abbott-85499
(Dexmedetomidine) to Human Serum Albumin (HSA) and
α_1-Acid Glycoprotein (AAG) at Normal Physiological Concentrations

Dexmedetomidine (ng free base/ml)	Sample	Percent [3H]A-85499 Bound[a] HSA[b]	AAG[c]	HSA/AAG[d]
0.85	1	80.65	65.22	81.94
	2	80.63	65.55	81.75
	Mean	80.64	65.39	81.85
8.5	1	80.83	63.97	82.49
	2	81.09	64.69	82.43
	Mean	80.96	64.33	82.46
21.25	1	80.39	63.85	82.01
	2	80.57	64.09	82.19
	Mean	80.48	63.97	82.10
42.5	1	81.24	62.48	82.24
	2	80.90	63.53	82.39
	Mean	81.07	63.00	82.32
85	1	80.47	62.97	82.34
	2	80.70	63.28	82.40
	Mean	80.59	63.12	82.37
0.85 - 85	Mean	80.75	63.96	82.22

[a] As determined by an ultrafiltration technique.
[b] Buffer solution (0.067 M phosphate, pH 7.4) containing 40 mg/ml human serum albumin.
[c] Buffer solution (0.067 M phosphate. pH 7.4) containing 0.8 mg/ml α_1-acid glycoprotein.
[d] Buffer solution (0.067 M phosphate. pH 7.4) containing 40 mg/ml human serum albumin and 0.8 mg/ml α_1-acid glycoprotein.

Summary:

[^{3}H] Dexmedetomidine was examined *in vitro* for binding to serum albumin and α-acid glycoproteins by an ultrafiltration technique. The concentation of dexmedetomidine was evaluated between 0.85 and 85 ng/ml and the concentrations of albumin and α_1-acid glycoproteins were varied to approximate clinical disease state conditions. The results indicate that dexmedetomidine may have slightly higher unbound levels in the disease states which substantially lower serum albumin levels. An elevated α_1-acid glycoprotein level can ameliorate this reduced binding and if these levels are reduced, the unbound fraction would be expected to further increase.

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[45]

Study Title: Abbott-85499 Drug Metabolism Report No.29 - Protein binding interactions between Abbott-85499-^{3}H (dexmedetomidine) and selected other drugs in human plasma (Protocol V97-034)

(#244)

Study No: Report No. R&D1971525
Vol #43, and page #182:
Conducting laboratory and location: Abbott Laboratories Division 46: Abbott Park, IL
Date of study report: Report No. R&D1971525

Methods: Blood was obtained from male and female voluteers who had fasted for at least 8 hours and had taken no medicine other than aspirin in the last week and no salicylates within the last 48 hours. The heparinized samples were centrifuged and the plasma frozen until use.

The protein binding was determined by an ultrafiltration technique. The solutions (as specified under Results:) were incubated at 37°C in shaking water baths and placed in a centrifuge devise that provided ultrafiltration during centrifugation. The filtrates were extracted with acetonitrile and analyzed chromatographically. The radioassay of the proteins was in a liquid scintillation analyzer.

Drug, lot#, radiolabel (if applicable), and % purity: Medetomidine HCl, with tritium on the bridge methyl group, was synthesized [redacted] nd the dexmedetomidine isomer was separated at Abbott by chiral chromatography. (Lot #55585-st-108; 66 Ci/mmol). The

Results:

The results are presented on the following table from the submission (v43/p184):

Compound	% A-85499-^{3}H Concentration	Bound
Control*	0.6 ng/ml	92.71
+ Fentanyl	3.0 ng/ml	92.61
+ Ketorolac	3.0 ug/ml	92.50
+ Theophylline	20.0 ug/ml	92.58
+ Digoxin	3.0 ug/ml	92.66
+ Lidocaine	6.0 ugiml	91.79

* = + 50% Ethanol

Summary:

The effects of fentanyl, ketorolac, theophylline, digoxin and lidocane on the *in vitro* protein binding of dexmedetomidine was minimal. There were no differences in binding as great as 1%.

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[46]

Study Title: Abbott-85499 Drug Metabolism Report No.30 - Effect of Abbott-85499 (dexmedetomidine) on the protein binding of selected other drugs in human plasma (Protocol V97-027)

(#245)

Study No: Report No. R&D/971526
Vol #43, page #201:
Conducting laboratory and location: Abbott Laboratories Division 46: Abbott Park, IL
Date of study report: September 1997

Methods: Unlabeled dexmedetomidine, 0.06 ug base/ml saline was the test concentration. Blood was obtained from male and female volunteers who had fasted for at least 8 hours and had taken no medicine other than aspirin in the last week and no salicylates within the last 48 hours. The heparinized samples were centrifuged and the plasma frozen until use.
The protein binding was determined by an ~~~~~. The solutions of plasma and test drugs were incubated at 37°C in shaking water baths for 15 minutes. The samples were then divided in two portions and one recieved an aliquot of normal saline and the other the unlabed dexmedetomidine. After an hour of additional agitation at 37°C, the samples were placed in a centrifuge devise that provided ultrafiltration. The filtrates were extracted with acetonitrile and analyzed chromatographically. The radioassay of the proteins was in a liquid scintillation analyzer.

Drug, lot# radiolabel, and % purity: Tritium labeled ibuprofen (10µg/ml), theophylline (20µg/ml), propanolol (0.02µg/ml), and digoxin (3ng/ml) were used and ^{14}C-labeled phenytoin (16µg/ml) and warfarin (10µg/ml) were also test solutions. Unlabeled dexmedetomidine (0.6ng/ml) (lot #2952609-0-AX). Radiochemical purity was ~~~~~

Results:
None of the tested compounds appeared to be significantly displaced by dexmedetomidine (0.6 ng/ml). There was no significant differences between the sexes. The combined results are presented on the following table from the submission (v43/p203).
Table 245-1:

Radiolabeled Drug	% Bound Control*	% Bound + Abbott-85499	% of Control
Phenytoin	93.55	93.53	100
Warfarin	99.38	99.37	100
Ibuprofen	99.55	99.55	100
Propranolol	75.47	75.36	99.9
Theophylline	60.99	60.69	99.5
Digoxin	33.75	34.46	102

* 0.6 ng/ml * + 50% ethanol

Summary:
Dexmedetomidine, at 0.6 ng/ml, did not displace any of the concomitant drugs from the plasma protein binding, *in vitro*. The mean Cmax, *in vivo*, during the use of the MRHID was 2.4 ng/ml, exceeding the test dose by 4-fold. This test may not be predictive of clinical effects.

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[47]

Study Title: Abbott-85499 Drug Metabolism Report No.22 -
In vitro determination of human red cell binding of Abbott-85499-3H (dexmedetomidine)
(Protocol V97-026) (#246)
Study No: Report No. R&D/97/371
Vol #43, page #221:
Conducting laboratory and location: Abbott Laboratories Division 46: Abbott Park, IL
Date of study report: September 1997
Methods: Blood was obtained from 2 male and 2 female volunteers who had fasted for at least 8 hours and had taken no medicine other than aspirin in the last week and no salicylates within the last 48 hours. The hemocrit was determined for each subject; M1=41.7%, M2=42.9%, F1=39.2% and F2=40%. The heparinized samples were spiked with labeled dexmedetomidine, 0.5 to 5ng base/ml, and incubated for 1 hour at 37°C. An aliquot of the samples was centrifuged at 4°C and 1 ml of plasma was extracted with acetonitrile and characterized ———
Drug, lot#, radiolabel, and % purity: Medetomidine HCl, with tritium on the bridge methyl group, was synthesized ———————the dexmedetomidine isomer was separated at Abbott by chiral chromatography. (Lot #55585-ST-108; 72.6 Ci/mmol, 278.3 mCi/mg) The

Results:
There was no metabolism of dexmedetomidine or degradation in the whole blood. There were minor concentration dependent differences in binding but the binding was low to the RBCs. There were no sex differences evident and the fraction bound to the red blood cells over the entire concentration range, 0.5 - 5 ng base/ml, averaged 0.183, the RBC to plasma concentration ratio was 0.325 and the whole blood to plasma ratio was 0.723. The following table was copied from the submission:

Parameter	Male	Female	Mean
f_{rbc}	0.180	0.186	0.183
C_{rbc}/Cp	0.302	0.348	0.325
C_{blood}/Cp	0.704	0.742	0.723

f_{rbc} = fraction bound; C_{rbc}= concentration in RBC; C_{blood}= concentration in whole blood
Cp= concentration in plasma

Summary:
The binding for dexmedetomidine to human red blood cells was low, only about 18% and this represented about 32% of the plasma concentrations.

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[48][45]

Study Title: Abbott-85499 Drug Metabolism Report No.27 - Conversion of [^{3}H]dexmedetomidine to [^{3}H] levomedetomidine in male subjects following a 2 µg/kg infusion of [^{3}H]dexmedetomidine HCl (#256)

Study No: Report No. R&D/97/458
Vol #45, page #153:
Conducting laboratory and location: Abbott Laboratories Division 46: Abbott Park, IL
Date of study report: August 1997

Methods: Plasma samples were obtained from human subjects (from study DEX-96-018), 10 minutes, 1, 2, and 4, 5 or 6 hours post administration of labeled dexmedetomidine, 2 ug/kg infusion. The samples were analyzed by [redacted] Some samples were spiked with labeled levomedetomidine to establish the minimum level of detection. The urine of the subjects was also analyzed for levomedetomidine was previously found *in vitro* to be rapidly glucuronidated.

Drug, lot#, radiolabel, and % purity: Medetomidine HCl, with tritium on the bridge methyl group, was synthesized . [redacted] and the dexmedetomidine isomer was separated at Abbott by chiral chromatography. (Lot #55585-ST-37 360 mcCi/ug) Unlabeled dexmedetomidine, Lot #295260-0-AX, was added to the labeled dexmedetomidine HCl and levomedetomidine (Batch PT0202). The radiochemical purity of [redacted]

Results:
Chiral conversion of dexmedetomidine to levomedetomidine was not detected in chiral chromatography of plasma samples from human receiving 2 ug/kg infusions. The limit of detection was 0.02 ng/ml. Reevaluation of data from study DEX-96-018 did reveal a possible exposure to the glucuronidated levo isomer. The possible exposure was slight, less than 0.5% of the AUC_{0-24} for total plasma radioactivity. The possible amount of glucuronidated levomedetomidine was less than 1.5% of the dose, in the 0-72 hour urine samples and it was present as an impurity of about 0.3% in the labeled dexmedetomidine administered.

Summary

If there is any chiral conversion of dexmedetomidine into the levo isomer, it is very slight and the levo isomer has been shown to be inactive and non-toxic at any feasable dose (see Toxicology, impurities).

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ADME - Pharmacokinetic Summary

Binding to blood proteins:
The *in vitro* study of plasma protein binding of dexmedetomidine in different species did not find any significant sex differences and monkey plasma had the least binding at about 85% with rats at 88% [34]. The dexmedetomidine binding was similar in dog, mouse and man, 93%, 95% and 94%, respectively. Dexmedetomidine was not extensively bound to human red blood cells over a range of concentrations. The fraction of dexmedetomidine bound averaged 0.183. The ratio of RBC concentration / plasma concentration averaged 0.325 [38]. The binding to $alpha_1$-glycoproteins was studied also. The concentration of dexmedetomidine was evaluated between 0.85 and 85 ng/ml and the concentrations of albumin and α_1-acid glycoproteins were varied to approximate clinical disease state conditions. The results indicate that dexmedetomidine may have slightly higher unbound levels in the disease states which substantially lower serum albumin levels [35].

The *in vitro* effects of other therapeutic agents on the plasma binding of dexmedetomidine was examined with fentanyl, theophylline, digoxin and other compounds and found to be minimal [36]. In the converse, dexmedetomidine did not affect the plasma protein binding of phenytoin, warfarin, theophylline or ibuprofen [37].

Absorption:
The absorption of dexmedetomidine from subcutaneous injection in the rat was rapid, the t_{max} was 0.6 to 0.7 hours [22], and the t_{max} in the rat after im injection was 0.33 hrs[47]. In the dog the intramuscular absorption was also rapid, t_{max} = 0.6 hours [23].

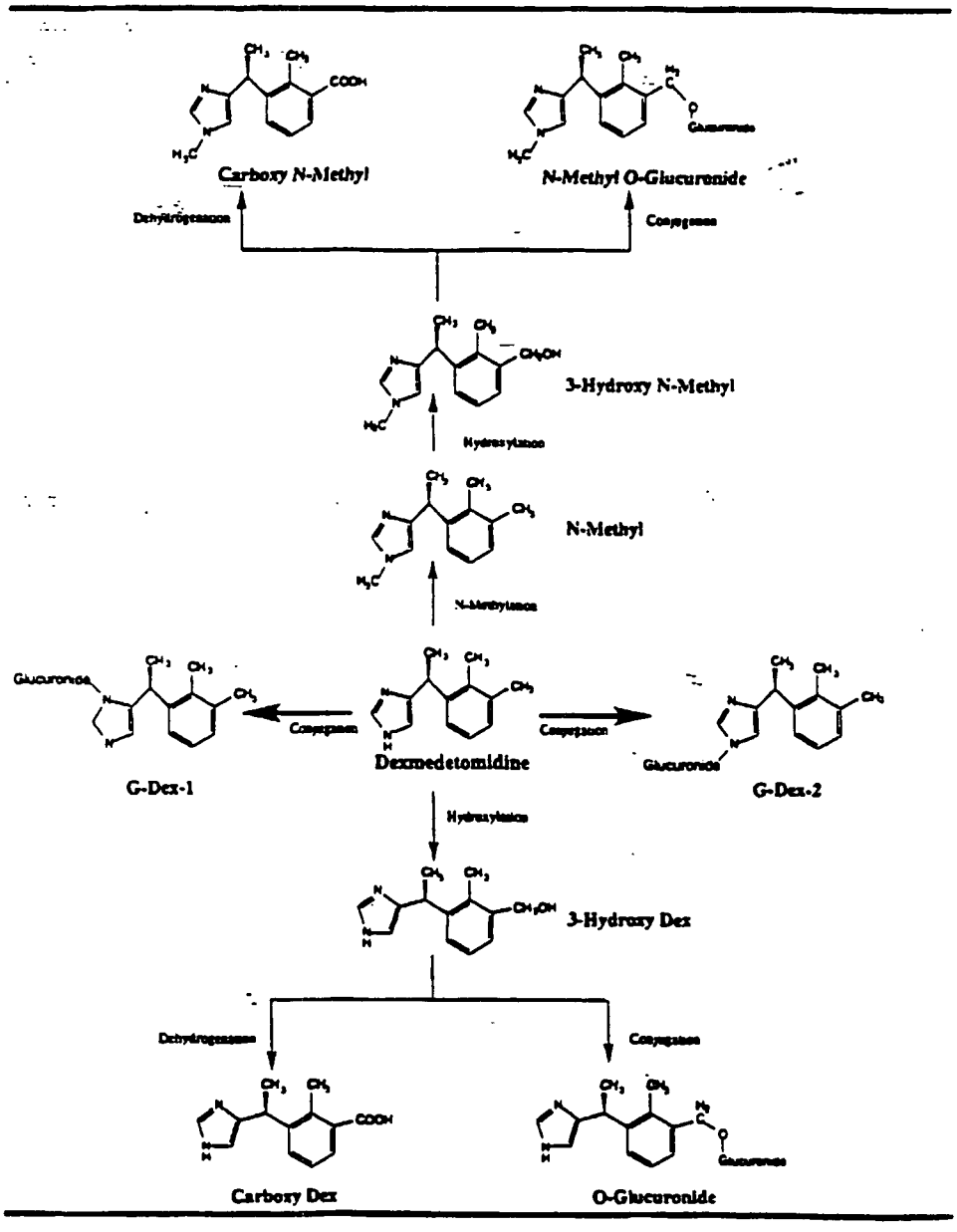
Distribution:
Tissue distribution in the rat was examined in one study the sponsor considered questionable, due to a specific activity too low to reliably measure the parent compound by HPLC [47]. However, the exposures of the adrenals, liver and kidneys after iv administration relative to plasma exposures (AUC values compared) was 51x, 34x and 15x, respectively.

Another study was submitted with the IND and previously reviewed (see attached Addendum 1, #239). In that study the sc dose of labeled dexmedetomidine (40 μg/kg) accumulated in the adrenals 72x plasma concentrations and 36-39x in the kidneys. The 20 μg/kg iv study [29] found that the labeled dexmedetomidine accumulated in the pigmented eyes of the Long Evans rats, but not the eye of the albino Sprague Dawley rats. However, the pigmented skin of the Long Evans rats did not accumulate the compounds. The exposure of various tissues to labeled compound exceeded the plasma exposure, as determined by AUC ratios. In the males, the exposure ratios were 185x, 54x and 11x, for adrenals, liver and kidneys, respectively. The tissue concentrations all exceeded the plasma concentrations at least at one time point. The mean peak concentrations in the brain were 6x greater than in plasma. The tissue levels of labeled compounds decreased significantly and after 72 hours the concentrations were only 0.1% to 5% of the peak levels.

Metabolism:
Dexmedetomidine is extensively metabolized in rats, dogs and humans as the parent compound is present as less than 1% of the administered dose in either feces or urine. The following discussion of primary metabolic pathways must be regarded as preliminary as the "other" in the metabolic tables represents 55% to 64% of metabolites in rats, 60+% in dogs and 30% of the urine metabolites in humans. The metabolism is similar in the different species as the first step involves N-methylation, bridge methyl hydroxylation, 3-methyl hydroxylation or N-glucuronidation. However, the N-glucuronidation is observed as a primary metabolite only in humans. The 3-methyl hydroxylation is favored in the rats and dogs but is also present in man. The glucuronidation of the 3-hydroxyl metabolite is a major pathway in rats and the oxidation of 3-methyl hydroxyl to the carboxy is a major pathway in rats and dogs, but minor in humans. The sulfate of the 3-hydroxy is a major plasma metabolite in rats and dogs but not in humans. While these metabolites have not been shown to be in active *in vivo*, they have been found weakly active in *in vitro* studies[3] and they represent 34% of the dexmedetomidine excretion[33]. The human plasma AUC for dexmedetomidine, 2 μg/kg i.v., is 3.26 and the AUCs' for the N-glucuronides are 7.8 and 1.37 [33]. This exposure of humans to the N-glucuronides is not seen in animals as the dog and rat do not make these metabolites except possibly in trace amounts. The proposed human metabolism is presented as a flow diagram on the following page.

Table of the proposed metabolic pathway in humans was copied from the submission (Vol43/pg071):

Figure 8. Proposed Dexmedetomidine Metabolic Pathway in Humans



The Pk parameters and the metabolites of rats, dogs and humans are presented in the following table from the submission (Vol 23/pg 299) :

Table Pk sum 1:

Summary of [^{3}H]Dexmedetomidine Metabolism in Animals and Humans -Plasma Data

Parameter	Rat	Rat	Dog	Dog	Human
Dose (ug/kg)	20. iv	20. sc	20. iv	20. sc	2.iv+
Plasma Total ^{3}H					
Cmax (ng Eq/mi)		2.48		8.98	
Tmax (hr)		2.13		6.0	
AUC(ngEq.hr/ml)	42.55	28.96	101.81	110.38	22.17
Dexmedetomidine					
Cmax (ng/mi)		2.03		4.39	
Tmax(hr)		0.50		2.0	
AUC(ng.hr/ml)	2.04	4.53	15.14	19.03	3.26
Metabolites					
AUC (ng Eq.hr/ml)					
Total ^{3}H	32.63	30.48	101.81	110.38	22.17
OH	0.62	0.46	1.01	1.00	nd
COOH	3.04	1.67	5.0	4.58	0.24
G-OH	3.05	1.96	8.37	5.94	0.67
SO_3OH	3.63	2.28	7.20	7.67	nd

Parameter	Rat	Dog	Dog	Rat	Human
GS-OH	nd	nd	nd	nd	nd
M-OH	nd	nd	nd	nd	nd
G-Dex-1	nd	nd	nd	nd	7.80
G-Dex-2	nd	nd	nd	nd	1.37
N-Me.	nd	nd	nd	nd	0.06
G-N-Me-OH	nd	nd	nd	nd	4.56
N-MeCOOH	nd	nd	nd	nd	0.07
H-3/D-7@	nd	nd	1.63#	4.60#	3.08#
Others	20.28	19.60	63.46	67.58	0.52
Reference	#238	#238	[32]	[32]	[33]

Dose= ng dexmedetomidine.HCl per kg
All data expressed as ng or ng Eq of free base
+= 10-minute infusion
nd = not detected

iv intravenous
sc subcutaneous
@= bridge hydroxy metabolite
corrected for loss of tritium

Elimination:

The elimination of dexmedetomidine in all species was rapid, $t_{1/2}$ less than 3 hours, and primarily in the urine. In human, 89% was found in the urine and 6% in the feces [46]. In another human study, metabolites in the urine represented 94% of the dexmedetomidine administered [33]. In bile-cannulated rats, urinary excretion amounted to 41% of the iv administered dose and 37% after sc administration [31]. In dogs, the urinary metabolites amounted to 79% of the iv dose and 81% of the subcutaneous dose [32]. The following tables depict the elimination of dexmedetomidine metabolites in various species and they were copied from the submission (V23/p295+):

Table Pk sum2:

Summary of Urinary Excretion of [^{3}H]Dexmedetomidine and Metabolites in Animals and Humans

Parameter	Rat	Rat	Dog	Dog	Human
Dose*	20, iv	20, sc	20, iv	20, sc	2, iv#
Urine (%Dose)					
Total ^{3}H	60.19	41.78	78.97	80.52	93.83
Dex.	0.88	0.40	nd	0.44	nd
OH	3.20	1.99	1.33	2.21	1.11
COOH	8.75	5.79	13.21	11.77	4.80
G-OH	8.32	6.50	10.82	8.06	7.66
SO_3OH	9.01	6.09	13.12	15.26	nd
GS-OH	nd	nd	nd	nd	nd
M-OH	3.34	2.68	nd	nd	nd
G-Dex-1	nd	nd	nd	nd	19.56
G-Dex-2	nd	nd	nd	nd	14.43
N-Me	nd	nd	nd	nd	nd
G-N-Me-OH	nd	nd	nd	nd	14.51
N-Me-COOH	nd	nd	nd	nd	3.76
H-3/D-7@	nd	nd	nd	nd	rid
Others	26.72	18.37	40.50	42.80	28.02
Reference	238	238	[32]	[32]	[33]

Dose ug dexmedetomidine per kg
iv = intravenous sc = subcutaneous
nd = not detected # = 10-minute infusion @ = bridge hydroxy metabolite

NDA 21-038

Table pK sum3:

Summary of Fecal and Biliary Excretion of [^{3}H]Dexmedetomidine and Metabolites in Animals and Humans

Parameter	Rat	Rat	Rat	Rat	Dog	Dog	Human
Dose'	20, iv	20, sc	20,iv	20,sc	20, iv	20, sc	2, iv*
Feces (% Dose)							
Total ^{3}H	33.23	47.66	ria	na	10.37	11.46	3.38
Dex.	nd	nd	na	na	nd	nd	0.06
OH	1.92	3.12	na	na	0.84	0.91	0.18
COOH	2.61	5.77	na	na	1.78	2.02	0.47
G-OH	2.36	3.64	na	na	nd	nd	0.04
SO_3OH	1.48	3.04	na	na	0.24	0.95	nd
M-OH	nd	nd	na	na	nd	nd	nd
GS-OH	nd	nd	na	na	nd	nd	0.09
G-Dex-1	nd	nd	na	na	nd	nd	0.01
G-Dex-2	nd	nd	na	na	nd	nd	nd
N-Me	nd	nd	na	na	nd	nd	nd
G-N-Me-OH	nd	nd	na	na	nd	nd	0.19
N-Me-COOH	nd	nd	na	na	nd	nd	nd
H-3/D-7@	nd	nd	na	na	nd	nd	0.06**
Others	24.87	32.10	na	na	7.52	7.58	2.28
Bile(%dose)							
Total ^{3}H	na	na	51.58	45.36	na	na	na
Dex.	na	na	0.05	0.05	na	na	na
OH	na	na	nd	nd	na	na	na
COOH	na	na	1.05	0.60	na	na	na
G-OH	na	na	14.96	15.26	na	na	na
SO_3OH	na	na	2.64	1.77	na	na	na
GS-OH	na	na	3.73	3.11	na	na	na
M-OH	na	na	0.26	0.37	na	na	na
G-Dex-1	na	na	nd	nd	na	na	na
G-Dex-2	na	na	nd	nd	na	na	na
G-N-Me-OH	na	na	nd	nd	na	na	na
N-Me-COOH	na	na	nd	nd	na	na	na
H-3/D-7@	na	na	nd	nd	na	na	na
Others	na	na	28.91	24.24	na	na	na
Reference	#238	#238	[31]	[31]	[32]	[32]	[33]

#238 = in original review Appendix 2 'dose = ng dexmedetomidine.HCl per kg

*=10-minute infusion iv = intravenous sc= subcutaneous **corrected for loss of tritium

nd = not detected na = not analyzed/not applicable @= bridge hydroxy metabolite

Table 5.8.17. Summary of Pharmacokinetic and Metabolism Results in Humans Given [^{3}H]Dexmedetomidine - Plasma Data (Males 2.0ug/kg)

Dexmedetomidine	AUC_{0-24} (ng Eq.hr/ml)	3.26
Metabolites		
AUC_{0-24} (ng Eq.hr/ml)		
OH		nd
COOH		0.24
G-OH		0.67
SO_3OH		nd
GS-OH		nd
M-OH		nd
G-Dex-1		7.80
G-Dex-2		1.37
N-Me		0.06
N-Me-OH		nd
G- N-Me-OH		4.56
H-2		0.54
H-3/D-7@		3.48#
N-Me-COOH		0.07
Others		0.52

Reference [33]

All values expressed as ng or ng Eq of the base/g
nd = not detected
@ = bridge hydroxy metabolite

Dexmedetomidine binds about 95% to plasma proteins in man, 93% in dog and 85% in mouse. It does not bind to red blood cells and there appeared to be little drug/drug interactions in plasma protein binding.

The distribution is rapid after iv administration and it was shown in the rat that all tissues accumulate enough dexmedetomidine to have tissue/plasma levels greater than 1 at some time point. The greatest accumulations were in the kidneys, liver and adrenals, with concentrations representing 11X, 54X and 185X the peak plasma level, respectively. The AUCs for total ^{3}H was 22.3 in the whole blood, 339 in kidneys, 750 in liver and 1145 in adrenals.

The human half-life was reported to be 2.6 hrs iv; the dog, 1.5 hrs iv in adults and 0.7 in juveniles; the rabbit, 1.83 hr iv and the rat, 0.7 hrs sc. The AUC (ng.hr/ml) in humans at 2 μg/kg iv was 3.26 and this compared favorably with the dog AUC of 15.14 at dose of 20 μg/kg, iv, and an NOAEL of 10 µg/kg/day. However, the rat at an NOAEL of 10μg/kg/day iv, a dose of 20

µg/kg had an AUC of only 2.04 ng.hr/ml. This does not provide a clear picture of exposure related toxicity. The AUCs in adult rats and dogs were approximately 3X those of juvenile animals, due mainly to the increased metabolic rate of the young.

The metabolism differs between species. The 3-hydroxylated, 3-carboxylated and the glucuronide of the 3-hydroxylated moieties appeared in the urine of all species tested. However, in the human, the glucuronides of the [redacted] and the N-methyl-hydroxy metabolites amount to 48.5% of the total dose administered and are not detected in the rats or dogs over a trace amount. The N-glucuronide metabolites, representing 34% of the total dose, have been shown to have weak α_2-agonist activity *in vitro*, but not *in vivo*, in limited testing. The lack of *in vivo* activity probably reflects a poor passage of glucuronides through membranes and into the CNS. These metabolites are not present in the rat or dog and therefore their pharmacology and/or toxicology have not been tested in the animal studies..

The excretion of dexmedetomidine and metabolites was mainly in the urine. e.g. 60% in rat, 79% in dog and 94% in human. The fecal excretion was 33% in rats, 11% in dogs and 3% in humans.

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TOXICOLOGY

[49]

Study Title: Subacute Toxicity Study of Dexmedetomidine (MPV-1440 x H.I.) by Daily Intramuscular Administration to Rats for 28 Days.
(#212)

Study No: TOX 90-001 (R&D/95/921)
Vol #33, and page #001:
Conducting laboratory and location: [redacted]

Date of study initiation: January 15, 1990
GLP compliance: yes, pg. 41: QA- Report: Yes pg. 26

Methods:
- species/strain: rats, Sprague-Dawley [redacted]
- #/sex/group or time point: 10 male and 10 female/ dose group
- age: about 28 days at initiation of treatment
- weight: 78-105 grams at start
- dosage groups in administered units: doses of 0, 20 (LD), 100 (MD) or 500 (HD) µg/kg for 28 consecutive days.
- route, volume: daily im bolus injections into thigh muscles of 0.5ml/kg.

Drug : Batch #OT 3841 [redacted]
Formulation/vehicle: dexmedetomidine in normal saline, no pH cited. Assays were done 3x in first week and once weekly thereafter. [redacted]
Observations and times:
Clinical signs: twice daily, AM after dosing and once PM in afternoon
Body weights: measured weekly
Food consumption: recorded weekly
Ophthalmoscopy: predosing and week 4 at end of dosing
Hematology: samples taken by heart puncture at study termination.
Clinical chemistry: at autopsy
Urinalysis: end of study , controls and high dose groups and 5/sex in low and medium dose groups.
Organ weights: from all animals at necropsy: liver, kidney, adrenals, lung, pituitary, brain, thymus, spleen and uterus and ovaries or testes, prostate and seminal versicles

Gross pathology: all gross lesions, brain (at least 3 levels), spinal cord (at least two levels), pituitary, salivary gland, mammary gland, heart, thymus, thyroid including parathyroid, lungs including bronchi, trachea, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, adrenals, pancreas, liver, kidneys, urinary bladder, aorta, ovaries, uterus, vagina, testes, epididymides, prostate, seminal vesicle, spleen, s.m. and mesenteric lymph node, femur, sciatic nerve, skeletal muscle (injection site), tongue and skin.

Histopathology:
In the control group and in the HD group, (500 ug/kg) of both sexes, if present:
All gross lesions, brain (including areas of cerebrum, cerebellum, midbrain and brain stem), pituitary, eyes, salivary gland, heart, thymus, thyroids including parathyroids, lung, trachea, esophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, adrenals, pancreas, liver, kidneys, urinary bladder, aorta, prostate, testes, epididymides, seminal vesicles, ovaries, uterus, vagina, spleen, s.m. and mesenteric lymph node, sciatic nerve, skin, mammary gland, tongue and skeletal muscle (injection site). Femur and spinal cord were not studied.

In the low (20 ug/kg) and medium (100 ug/kg) dose group of both sexes: All gross lesions, eyes, heart, thymus, lung, adrenals, liver, kidneys, prostate, testes, epididymides, seminal vesicles, ovaries, uterus, vagina, spleen, s.m. and mesenteric lymph node and injection site.

Results:
Clinical signs:
Dose dependent transient piloerection and sedation were observed in the mornings after dosing and in the afternoon, occasional MD animals showed slight sedation. Exophthalmia was observed in mid and high dose group after dosing. The HD demonstrated only slight sedation and/or piloerection in the afternoon observations.

Body weights:
Males: The body weight gain of the high dose group was 76% of the control groups gain and in the medium dose group, the weight gain was reduced 13% from control at autopsy. The low dose group had only an insignificant 6% reduction from control weight gain at autopsy.

Females: The high dose group gained only 85% of controls at autopsy and the mid and low dose groups weight gains were marginally reduced, 4 and 6%, respectively.

Food consumption:
The high dose group males ate a total of 19% less than controls, the medium dose, 11% and the low dose group, -6%. The reported ANOVA was not significant for dose but was for the dose.time variable. However, further analysis did not find any significant difference between controls and any dose group.

Ophthalmoscopy:
Controls: slight white focus in one eye of one male.
Low dose (20µg/kg/day): 2 males with minimal corneal opacity in right eye.

Mid dose (100 µg/kg/day): 6 males with minimal corneal opacity in one or both eyes, 1 male with moderate corneal opacity in one eye and slight in the other. 8 females with minimal corneal opacity in one or both eyes.

High dose (500µg/kg/day): All ten males and females with minimal to moderate corneal opacity in both eyes and 7/10 with opacity severe enough to prevent fundus observation.

Hematology:
No dose group had hematology parameters that were statistically significant in comparison to control, but the high dose group males had a small decrease in the RBC's (7.73 vs 7.34) and slight increase in the percentage of neutrophils (5.0 vs 8.5♂: 4.8 vs 7.0 ♀) and a slight, but significant decrease in lymphocytes (91.4 vs 85.2♂: 91.7 vs 88.7♀). There no significant changes in any parameters in the females of any treatment group. The hematological changes were small and probably of no toxicological significance.

Bone marrow: Only the controls and high dose groups were examined as the observed changes were not of toxicological significance. There was a statistically significant, but considered of no toxicological significance, increase in eosinophils (2.59 vs 3.81) in the males and segmented neutrophils in males and females, 0.64 to 0.97 and 0.61 to 1.05, respectively.

Blood coagulation time:
The prothrombin times did not change significantly and the statistically significant change in SPA(Sago prothrombin assay), from 3.54 seconds in control males to 3.62 seconds in the 500 µg/kg/day group, was not of toxicological significance. Similar changes were seen in the high dose females.

Clinical chemistry:

Variable	sex	control	20 µg/kg/day	100 µg/kg/day	500µg/kg/day
sodium	♂	154.1	153.8	152.3	150.6*
	♀	150.2	149	149.7	150.4
potassium	♂	6.11	6.17	5.85	5.53*
	♀	5.69	5.66	5.84	5.78

Variable	sex	control	20 µg/kg/day	100 µg/kg/day	500µg/kg/day
Calcium	♂	2.97	2.91	2.90	2.89
	♀	2.88	2.77	2.83	2.76*
phosphate inorganic	♂	3.76	3.50	3.21*	3.27*
	♀	3.28	3.19	3.15*	3.10*
protein	♂	4.23	4.21	4.20*	4.18*
	♀	4.24	4.21	4.16*	4.19*
creatinine	♂	3.95	3.88	3.85*	3.84*
	♀	3.95	3.96	3.89*	3.88*
cholesterol	♂	2.32	2.30	2.22	2.49
	♀	2.93	2.63	2.18*	2.02*
Alk phos	♂	507.4	606.3	607.5*	647.8*
	♀	325.6	342.0	421.9*	555.2*
LD	♂	835	572	338*	342*
	♀	782	824	537*	529*
CK	♂	408	339	236*	260*
	♀	340	301	234*	228*

The decreased cholesterol, serum proteins and inorganic phosphorus may all be related to the dose related reduction in body weight gain and reduced food consumption. The elevation of Alkaline Phosphatase was less than 2 fold even at the high dose may only represent the gastrointestinal isozyme and has no toxicological significance. The lack of clinical relevance is also seen with reductions on lactate dehydrogenase and creatinine kinase. The major source of creatinine and creatinine kinase is striated muscle and reduction in serum levels is probably related to the sedative effects and hypoactivity produced by dexmedetomidine.

Urinalysis:

A dose-related decrease in urine volume was observed only in the males and urine osmolarity was increased in the high dose males. The osmolarity was not measured in the low and mid dose groups. Because glucose was found in the urine of all the high dose group, both ♂ and ♀, the low and mid dose groups(5/sex/dose) were also tested for urinary glucose and was present in all the mid dose animals tested. The glucose was tested by dip strip and not quantified. The pancreas

was examined for histopathology in the controls and the high dose group and none was observed. However, the glucosuria was probably not due to insulin deficits as the glucose blood levels of the treatment groups had slight, dose related, decreases in blood glucose levels, not statistically significant. The kidneys were examined histologically and there was no pathology that didn't include the control group or occurred in only one or two of the dexmedetomidine dose group. This glucosuria was most probably a pharmacological effect of the α_2 agonist effects of dexmedetomidine as this has been observed after clonidine induced elevation in blood glucose[1,2,3]. The elevated blood glucose was not observed as the blood sample was taken probably about 24 hours after last dexmedetomidine injection and the α_2 stimulated glucose rise lasts less than six hours at a bolus dose of 30 µg/kg [5]. The α_2 agonist clonidine was shown to induce glucosuria, after the induced blood glucose rise, by an increase in the GFR and "filtered fraction"[4]. This is a pharmacological effect, is transient, requires large bolus doses and produces no end-organ toxicity.

Organ Weights:
The absolute organ weights were slightly decreased and this would be expected with the dose related reduction in body weight gain. The organ weights relative to the body weights gave a different picture. The relative lung weights were slight increase at the high dose in both sexes. The relative brain weights were slightly increased in both sexes in a dose dependent manner. In the males, the relative adrenal weights were increased in the high dose group and in the females, the relative kidney weights were slightly increased in the high dose group. In the males, the prostate and seminal vesicles were slightly decreased in the high dose group and the females in this group had slightly reduced relative uterus and ovary weights.

Gross pathology:
The cloudy cornea were evident in both sexes in the medium (90%♂, 80% ♀) and high dose (100% in both sexes) groups.

Histopathology:
In the adrenals, hypertrophy of the zona glomerulosa was evident at medium dose, 5/10 males, and at the high dose, 6/10 males and 4/10 females. This was cited as a pharmacological effect as α_2 stimulation of renin release and subsequent adrenal mineralocorticosteroid production.

Appearance of perivascular hemosiderin laden macrophages in the lung were dose related. At the low dose, 3/10 males were observed with minimal numbers and at the medium dose, 9/10 males and females had minimal numbers. At the high dose, 4/10 males and 10/10 females were minimal, 5/10 males were slight and 1/10 demonstrated a moderate number of hemosiderin laden macrophages. The lung does not appear, however, to be site of dexmedetomidine toxicity for other lung pathology was not drug related. Focal chronic inflammation was seen in 1/10 males in control group and 3/10 females and the only observations in the treated groups was 1/10 in both males and females in the low dose group.

Keratitis of the eyes were observed at the medium dose and high dose groups of both sexes. The severity ranged for minimal to moderate and increased in severity with dose. This corresponded to the observed cloudy corneas.

Upon examination of the injection sites, focal regenerative muscle fibers were observed. In the control group, minimal in 5/10 ♂and 4/10♀; at 20 µg/kg/day, 4/10 ♂ and 4/10♀; at 100 µg/kg/day, 5/10♂ and ♀ and 1/10 in both sexes was graded as slight. At the high dose, 500 µg/kg/day, 7/10♂, 6/10♀ were minimal, 2/10♂ and 1/10♀were slight and 1 male exhibited a focus of fibrosis.

In accordance with the reduced relative weight of the seminal vesicles in the high dose males, 3/10 had immature vesicles and 2/10 had immature epididymis, testis and reduced acinar size of the prostate. The prostate and seminal vesicle effects were observed in 2/10 in the medium dose group. This could be attributed to the reduced weight gain and delayed sexual maturation although this could be due to α_2 stimulation alteration of the hypothalamic-pituitary axis as seen with clonidine and medetomidine.

Summary

The daily bolus im injection of dexmedetomidine at 20, 100 and 500 µg/kg/day in rats for 28days produced a minimal extent of end-organ toxicity and no mortality. The most evident effect was a dose-related corneal opacity with keratitis, probably due to the pharmacologically reduced tear production and loss of blink reflex. The cloudy cornea was observed in 2/10 males in the low dose group and all other treated animals, but is a pharmacological consequence of reduced pain sensation and eye lubrication. There was significant reduction in body weight gain, -24% and -13% in the high dose group and -15% and -4% in the medium dose groups, males and females, respectively. The food intake was reduced and numerous organs were significantly lighter in the treated animals. However, organ weights relative to body weights indicated an increase in relative lung and brain weight and a decrease sex organ weights. The latter was collaborated histologically as immature testis, epididymis and prostate were observed in the high dose males. This could be due to the reduced body weight gain and/or α_2 stimulation disruption of the hypothalamic-pituitary-adrenal and gonadotropin axis as has been seen with clonidine. Minimal hypertrophy of adrenal zona glomerulosa was observed in males at 100 and 500 µg/kg/day doses and only at the higher dose in females. This was attributed to the dexmedetomidine stimulation of renin release and the subsequent increase in mineralocorticoid synthesis and release.

No toxicologically significant hematological changes or clinical chemistry changes were observed and the statistically significant increase in prothrombin time in males from 3.54 to 3.62 seconds was not toxicologically significant. The glucosuria, observed in all mid and high dose animals tested, was most likely a pharmacological effect of α_2 stimulation. There were some hemosiderin containing perivascular macrophages in the lungs of males in all treatment groups

and in the lungs of the females in the two high dose groups. There were no other treatment related histological changes in the lungs and the significance of the hemosiderin contatining macrophages is not known. The decreased body weight gain, decreased organs weights, cloudy corneas and macrophages in the lung at the 100 and 500 µg/kg/day groups were drug treatment related. The NOAEL was the LD, 20 µg/kg/day. This provides a safety ratio of less than 1.0 in comparison to the MRHID in mg/m^2. The LOAEL was 100 µg/kg/day and on a a mg/m^2 basis, was 3X greater than the MRHID.

References:

1) Gotoh, M, Iguchi A, Sakamoto N. "Central versus peripheral effect of clonidine on hepatic venous plasma glucose concentrations in rats" Diabetes 37(1):44-9(1988)

2) DiTullio NW, Cieslinski L, Matthews WD, Storer B. "Mechanisminvolved in the hyperglycemic response induced by clonidine and other alpha-2 adrenoreceptor agonists" J. Pharmacol Exp Therap 228(1):168-73 (1984)

3) Moratinos J, Carpene C, de Pablos I, Reverte M. "Role of alpha 1- and alpha 2-adrenoreceptors in catecholamine-induced hyperglycemia, lipolysis and insulin secretion in conscious fasted rabbits" Br J Pharmacol 94(2):299-310 (1988)

4) Biollaz J, Roch-Ramel F, Kirchertz EJ, Atkinson J, Peters-Haefeli L. "The renal effects of clonidine in unanestetized rats" Eur J Pharmacol 58(4):407-18(1979)

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[50]

Study Title: Twenty-eight day Intravenous Toxicity Study of Dexmedetomidine Hydrochloride in Rats (#213)
Study No: TOX-89020 R&D/95/915
Vol #34, page #001:
Conducting laboratory and location:
Date of study initiation: November 1989 - December 1989
GLP compliance: Yes pg 23
QA- Report Yes (x) No ()
Methods:

- species/strain: Rats/Sprague Dawley: 10/sex/treatment group: age, 28 days
- weight: 82-104 g at start
- dosage groups in administered units: 0, 10, 40 and 160 mcg/kg; control, LD, MD, HD, respectively.
- route, volume: iv bolus injection in tail vein, daily for 28 days

Drug, lot#, % purity: Batch #OT 3841
Formulation/vehicle: formulation made daily; dexmedetomidine hydrochloride in normal saline; Post use verification done on 9 occasions and 93-105.7% of intended concentration except for Day 3 of dosing when 10 and 40 mcg/kg doses were 65% above intended due to dilution error.
Observations and times:
Clinical signs: recorded twice daily, after morning dosing and again in afternoon
Body weights, Food consumption, and Ophthalmoscopy: weekly
Hematology: at autopsy; Hb, PCV, RBC, WBC, WBC differential, Platelet count, PTT, MCH, MCV, MCHC.
Clinical chemistry: standard screen and blood glucose.
Urinalysis: volume, pH, osmolality, protein, glucose, ketone, blood and sediment.
Organ weights: liver, kidneys, adrenals, pituitary, brain, thymus, heart, lung, spleen, uterus and ovaries/testes, prostate and seminal vesicles.
Gross pathology: all tissues and followed with histopath if observed
Histopathology: Any gross lesions and all standard tissues in control and high dose groups.

Results:
Clinical signs: Dose dependent sedation and piloerection was observed in dexmedetomidine treated animals and exophthamia was observed in the high dose group. No mortality was observed.
Body weights: slight decrease in HD weight gain, -9.3% in males and -4.2% in females but statistically significant in both sexes.
Food consumption: no significant difference
Water consumption: The HD males drank 25% more water than controls and the HD females 27% more water, significant in both cases.
Ophthalmoscopy : In LD no changes seen. In MD minimal corneal opacity seen in a few of both sexes and in HD minimal to moderate corneal opacities in most animals.
Hematology: There were significant differences in a number of parameters, in both sexes, mainly in the HD group. The statistically significant reduction in RBC in both males (-7.4%) and females (-4.7%) was not considered to have toxicological significance. There was a significant decrease in PCV and Hb concentration in HD males, small and not of toxicological significance. The HD females had significant reductions in MCH and MCHC.
Bone Marrow: The HD males had a 25% increase in eosinophils and the HD females a 24% increase. The sponsor contends that this increase is so small as to be inconsequential and this is consistent with the fact that there were no significant increase in WBCs in the peripheral blood.
Clinical chemistry - There were slight but significant decreases serum Na, potassium, magnesium and protein in both sexes at the HD and Na in the MD groups. These slight changes probably reflect the increased water consumption in the HD groups. There were significant, but small, increases in alkaline phosphatase in MD and HD animals of both sexes. The animals were young and this increased AFOS may be reflecting an isoenzyme from the bone development. The

decreased serum cholesterol and creatinine kinase in HD males and females, although statistically significant, probably has no toxicological significance. See table below.

Urinalysis - A significant finding was the presence of glucose in the urine of some males and females at the MD and nearly all at the HD. No glucose was found in the urine of control animals; see following table:

Changes from control values in Clinical Chemistry and Urinalysis										
Percent change from control values - statistically significant							Urinalysis - obvious changes			
	S-cho		S-AFOS		S-CK		Urine -glucose present			
	M	F	M	F	M	F	M	F	M	F
MD	-17		+10	+39			1/4	2/4	control	
HD	-15	-20	+12	+36	-31	-32	10/10	9/10	0/10	0/10

Histopathology:

Necropsy - treatment group differences				
	Adrenal hypertrophy zona glomerulosa		Lung - hemosiderin in macrophages	
	M/10	F/10	M/10	F/10
control	0	0	0	0
LD	0	0	7	1
MD	0	3	10	6
HD	6	4	10	10

Relative Organ Weights: The HD males and females had relative thymus weights significantly less than control by 17% and 12%, respectively. The relative brain weights were significantly greater than controls in the HD group, 11% in the males and 4.5% in the females. The relative liver weights of HD females were 12.5% greater than controls, and the difference was statistically significant, which was not true for HD males. However, histopathology finding in the livers of HD females (V34/pg54) were less than controls and for the HD males (Vol. 34/pg 141), often had focal necrotizing inflammation, 6/10, and 2/10 for HD females.

Summary

Ten rats/sex/dose were injected in the tail vein daily for consecutive 28 day with dexmedetomidine hydrochloride at doses of 0, 10, 40, and 160mcg/kg/day. No mortality was observed. Dose related sedation and piloerection was observed transiently after dosing and at the HD, exophthalmia was observed. The HD animals had less than 10% reduction in body weight gain and there were no differences in food intake. The high dose animals did drink significantly more water (+25-27%). The serum concentrations of sodium, potassium and magnesium were slightly reduced in the HD animals, possibly due to the increased drinking.

Glucosuria was evident in nearly all the HD group, few in the MD and none in LD or control groups. This is an expected effect of α_2 agonists as clonidine has been found to increase blood glucose[1,2,3,4] and induce glucosuria by an increase in the GFR and "filtered fraction"[4]. The increased blood glucose by dexmedetomidine was reported earlier [5]. This elevated blood glucose most likely occured in this study, but the blood sampling was about 24 hours after the last dose and the blood glucose rise lasts less than 6 hours after a 30 μg/kg bolus dose [5].

There were insignificant increases in blood WBCs, but the bone marrow eosinophils were significantly increased in both sexes at the HD of 160 mcg/kg/day. There was a dose related hypertrophy of the adrenal zona glomerulosa and hemosiderin containing macrophages in the lungs. In the HD females there was a significant increase in relative liver weights, but not in HD males.

The sponsor chose the MD, 40 mcg/kg/day, as the No-toxic-effect dose. The glucosuria in some of the MD animals is quite probably a phamacological action that has been seen at even lower doses and is not toxicologically significant, but the corneal keratitis, elevated S-ALD, adrenal hypertrophy and increased lung hemosiderin-laden macrophages at 40 mcg/kg/day, indicates 10 mcg/kg/day is the NOAEL. This has a ratio of less than 1.0 with MHRID in terms of doses in mg/m^2.

References:

1) Gotoh, M, Iguchi A, Sakamoto N. "Central versus peripheral effect of clonidine on hepatic venous plasma glucose concentrations in rats" Diabetes 37(1):44-9(1988)

2) DiTullio NW, Cieslinski L, Matthews WD, Storer B. "Mechanisminvolved in the hyperglycemic response induced by clonidine and other alpha-2 adrenoreceptor agonists" J. Pharmacol Exp Therap 228(1):168-73 (1984)

3) Moratinos J, Carpene C, de Pablos I, Reverte M. "Role of alpha 1- and alpha 2-adrenoreceptors in catecholamine-induced hyperglycemia, lipolysis and insulin secretion in conscious fasted rabbits" Br J Pharmacol 94(2):299-310 (1988)

4) Biollaz J, Roch-Ramel F, Kirchertz EJ, Atkinson J, Peters-Haefeli L. "The renal effects of clonidine in unanestetized rats" Eur J Pharmacol 58(4):407-18(1979)

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[51]

Study Title: Dexmedetomidine Hydrochloride: Subacute Toxicity Study by Daily Intramuscular Administration to Male Dogs for Four Weeks. (#215)
Study No: TOX-90006, R&D/97/116
Vol # 35, page #190:
Conducting laboratory and location: ([redacted] d
Date of study initiation: March 29, 1990
GLP compliance: yes, pg. 205
QA- Report Yes (x) No () pg.206
Dosing:
- species/strain: Beagle dogs
- #/sex/group: 3 males/dose
- age: 10 to 27 months at start
- weight: 9.3-14.8 kg
- dosage groups in administered units: 0, 10, 50, 250 µg/kg/day; control, LD, MD, HD
- route: im, gluteal or femoral muscles of hind legs, alternated daily injections at 0.05 ml/kg

Drug, lot#, radiolabel, and % purity: batch QTO231
Formulation/vehicle: dexmedetomidine hydrochloride dissolved in normal saline, pH not cited
Observations and times:
Clinical signs: at least 3 times daily
Body weights:weekly
Food consumption: weekly, 400 g food offered / day, remains weighed in morning.
Ophthalmoscopy: prior to testing and at end of last dosing week, without dialation at beginning of each week of testing.
Hematology: pretesting, week -1, and 6th day of 4th week of testing:
Clinical chemistry: pretesting, week -1, and sixth day of 4th week of testing and some day1 of week 3.
Urinalysis: during week 4 from metabolism cages
Organ weights: Gross pathology:
Organs weighed:
Histopathology: All at necropsy
Hematology:
The samples were collected into EDTA tubes. The following parameters were determined using a Coulter Counter ZB: red blood cell count. white blood cell count and platelet count. In addition, packed cell volume was determined using a hematocrit centrifuge. Hemoglobin was determined by the cyanmethemoglobin method using a [redacted]. Differential white blood cell count (May-Grunwald-Giemsa stain) and reticulocyte count (Brilliant Cresyl Blue stain) were determined microscopically. The red blood cell indices (MCH, MCV and MCHC) were calculated.

The samples for blood coagulation studies were collected into citrate tubes. Stago prothrombin complex assay and partial thromboplastin time were measured using BE-Coagulator.

Clinical Chemistry:
Samples were collected into serum tubes. The following parameters were determined using [redacted] alyzer: sodium, potassium, chloride, calcium, magnesium, phosphorus (inorganic), protein, albumin, creatinine, urate, urea, total bilirubin, cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, creatinine kinase and lactate dehydrogenase. Globulin content was calculated (total proteins - albumin). Blood glucose was determined using the glucose oxidase method (EDTA blood). Blood glucose was analyzed at the study site, and all the other analyses were made by the clinical laboratory [redacted]
Urinalysis
The urine samples were collected from all animals during week 4 using metabolism cages. The following parameters were determined: appearance volume, osmolality and sediment (microscopy). Semiquantitative analysis using Labstix® determined values for pH, protein, glucose, ketones and blood.
Necropsy:
On the completion of 4 weeks' treatment the dogs were sacrificed over a period of two days. The dosing of the individual treated animals was continued until the day prior to sacrifice. The duration of the dosing period is reported as being 4 weeks.
The macroscopic external and internal appearance of the tissues was noted and any abnormalities recorded. The following organs from all animals killed at the scheduled sacrifices were dissected out and weighed: adrenals, brain, heart, kidneys, liver, lungs, pituitary, spleen, thyroids (with parathyroids), prostate and testes. The relative weight of organs was calculated by dividing the organ weight (kg) by the bodyweight (kg) at autopsy and multiplied by 100.
All gross lesions, brain (including areas of cerebral cortex, thalamic nuclei. mid-brain, medulla and cerebellum), spinal cord (cervical, thoracic and lumbar regions), pituitary, eyes, submandibular salivary gland, heart, thymus. thyroids including parathyroids, lung, trachea, esophagus. stomach (body and antrum), duodenum, jejunum, ileum, caecum, colon rectum, adrenals, pancreas, liver, gall bladder, kidneys, urinary bladder, aorta (arch and abdominal), testes, epididymus, prostate, spleen, lymph node (submandibular and mesenteric), sternum (with marrow), femur (with articular surface), sciatic nerve, mammary gland, skin, tongue, skeletal muscle (biceps femoris) and injection sites.
Histopathology:
The tissues listed above (except sternum and femur) from all animals were examined by light microscopy. Prior to microscopic examination, tissues were embedded in paraffin wax and sections cut at 4 micrometres and stained with hematoxylin and eosin.
Bone marrow examination:
The bone marrow smear was taken from the femur of all animals during necropsy and was stained with May-Grunwald and Giemsa for microscopical examination. The percentage of

different cell types as well as the M/E ratio was calculated.

Results:
Clinical signs: No mortality occured. Dose-related sedation with durations of 1-2hr LD, 3-5hr MD, and 5-7hr HD. Slight sedation in the LD group and full sedation in all dogs in the MD and HD groups. In the HD group, muscle twitching, reddish eyes and irregular respiration or increased respiratory rate, were seen intermittantly.

Body weights:
The control dogs gained an average of 28 gms and HD group lost an average of 79 grams and this was statistically significant.
Food consumption
Each dog was offered 400 grams of dry food each day and all dogs consumed the entire amount. However, the because the HD group started 81 grams heavier, the restricted diet would probably have more effect on this group, but the 5-7 hours of dexmedetomidine induced sedation daily probably also had an effect.
Ophthalmoscopy
The HD group had small areas of hazy/opacity on the cornea, unilaterally. At the end of treatment, one of the dogs had recovered and because the lesions were reversible and unilateral, the sponsor attributed this to reduced tearing and long-lasting corneal dryness during the prolonged sedation period each day.

Hematology
There was a slight but significant increase in WBC in HD group as compared to control dogs, but still within the normal range and not considered treatment related by the sponsor. No other statistically significant differences were observed. RBC's, hemoglobin, platelet count, differential white blood cell count or RBC indices, MCH, MCV, MCHC.

Clinical chemistry
Creatinine significantly decreased slightly in the MD and HD groups, at both 3 and 4 weeks of testing. The toxicological significance is probably minimal as the sponsor states.

Alkaline phosphatase significantly increased in the MD and HD. MD = 1.6x and 1.8x at 3 and 4 weeks and HD = 2.6x and 1.7x at week 3 and 4 respectively. AST, aspartate aminotransferase, also increased slightly, but significantly in the HD, 1.4x and 1.2x at week 3 and 4.

The mean serum Alanine amino transferase (ALT) of the HD group, was increased significantly, 5.2x and 4.4x at week 3 and 4, respectively. However, at 3 week one of HD had a more than 8x its basal level of ALT. Creatine Kinase was slightly, but significantly elevated on week 4, 1.4x in the HD dogs. The changes were minimal, except for ALT mean elevation close to 5 fold in the HD group, at both 3 and 4 weeks. This indicates some dose related hepatotoxicity.

Urinalysis
There were no significant findings in the urinalysis and this included no glucose, keto bodies or blood in any of the dose groups or in control dogs.

Histopathology
Intracytoplasmic eosinophilic inclusions in the hepatocytes were evident in all three dogs in HD group and 1/3 in MD group. This evidence of hepatotoxicity was not observed in any of LD or control dogs.

At the site of injection there was evidence of regenerative muscle fibers in all groups. The presence was minimal in 2/3 controls, 1/3 LD and 2/3 MD. However, in the HD group 1/3 was slight and 2/3 were moderate and all three had focal fibrosis.

The appearance of the brown pigmentation in the kidney tubules, regenerative tubular hyperplasia and degenerative tubules were observed sporadically in the controls and all dose groups and the findings are considered incidental, not indications of drug related renal toxicity.

Key Study Omissions:
There was no Pathology Report on the low dose or control dogs although some histopathology data was cited in the results. There were no tables of relative organ weights although they were included in the protocol. There was no description of what the term sedation actually meant as was there a loss of righting reflex or only slow movement and closed eyes? The term "full sedation" probably was loss of righting-reflexd, but was this the entire 3-5 hours of sedation in the MD group?

Summary

The high intramuscular dose (HD) in male dogs, 250 µg/kg/day of dexmedetomidine, produced extensive sedation lasting 5-7 hours. Muscle twitching, redness of eyes and irregular breathing were observed. The liver damage was evident in histopathology with eosinophilic inclusions and in the clinical chemistry, ALT mean levels were elevated about 5-fold at both 3 and 4 weeks of treatment and one of HD dogs was elevated more than 8-fold on week 3 and about 7-fold on week 4. The elevation of ALD levels was near 2-fold at both 3 and 4 week in the HD dogs and Alkaline Phosphatase was elevated 2 to 3-fold in the HD group and 2-fold in one dog of the MD group in week 3. The hepatotoxicity is also evident in this MD dog (#8) by histology which reported intracytoplasmic eosinophilic inclusion in the liver tissue.
The MD, 50 µg/kg/day, also was sedative and this sedation lasted 3-5 hours.
The LD group, 10µg/kg/day, was sedated generally scored slight on sedation, but this lasted 1-2 hours. There was no elevation of liver enzymes or hematology changes, there was no Pathology Report on the individual LD or Control group animals, but the 10µg/kg/day was most probably the NOAEL.

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[52]

Study Title: Dexmedetomidine Hydrochloride: Subacute Toxicity Study by Daily Intramuscular Administration to Female Dogs for Four Weeks. (#216)

Study No:TOX 90-013

Vol # 36, and page #:15

Conducting laboratory and location: [redacted]

Date of study initiation: May 3, 1990

GLP compliance: Yes pg 16

QA- Report Yes (x) No ()

Methods:

Dosing:

- species/strain: Beagle dogs from [redacted]
- #/sex/group: 3 females/dose
- age: 11 to 23 months at start
- weight:8.23-11.9 kg
- dosage groups in administered units: 0, 10, 50, 250 µg/kg/day; control, LD, MD, HD
- route, form, volume, and infusion rate: im, gluteal or femoral muscles of hind legs, alternated daily injections at 0.05 ml/kg

Formulation/vehicle: dexmedetomidine hydrochloride dissolved in normal saline, pH not cited

Hematology, Clinical Chemistry, Urinalysis, Necropsy, Histopathology, Bone marrow examination and Observations and times were same as above study in male dogs.

Results:

Clinical signs:

Sedation was seen in all treated dogs, in MD and HD dogs, sedation was seen after every injection. The duration of sedation was dose dependent and was 2-3 hrs in LD, 3-4 hrs MD and 5-7 hrs HD. Muscle twitch, salivation and irregular respiration was seen occasionally in the HD group.

Body weights

A slight, non-significant weight loss was seen in the HD group.

Food consumption

During the first 2 week, the controls, LD and MD dogs all ate the entire 400 g offered. The third week the portions were 500 g and although the weeks supply should have been 3500 g (7x500), seven of the 12 dogs ate 2900 g. One dog in HD group (#10), ate less than offered food during every week and only 1547 g on week three.

Ophthalmoscopy
In control dogs, #3 was observed to have localized hyperreflexia in the right eye prior to start of study and again on last day of study, not on weeks 2 or 3. The dog #3 also had mucus discharges for the right eye on the 4th day of week 4. In the LD group, dog #4 had a central opacity of 2mm prior to start and weeks 2 and 3. During weeks 3 and 4, the central opacity was just observable at 1 mm. In the MD group no abnormal findings were observed. In HD group, dog #11 had bilateral central opacities, just observable weeks 2,3 and 4. Dog #12 had just observable opacities the 4th and 7th day of week 4. The opthalmologist attributed this to the eye dryness and prolonged sedation.

Hematology
There were no toxicologically significant changes, although statistically significant increase in RBC counts were observed in MD group and neutrophil percentages increased in LD and MD groups. The changes were all within normal ranges.

Clinical chemistry
There was a slight but significant decrease in creatinine levels in the MD and HD groups. This was not considered significant toxicologically. There were also statistically significant changes in potassium, chloride, calcium, inorganic phosphorus and albumin among one or more of the treatment groups, but all these changes were within normal ranges and were considered incidental.

The Alanine amino transferase (ALT) values were not significantly different between groups, but this was mainly due to control dog #1 which had high initial values about 2x the 3 week values. Actually 2/3 of the HD group had elevated level on week 3 and 4, 3.2 to 6-fold their baseline levels. One of these HD dogs also had GTT levels which more than doubled on week 3 and was still elevated on week 4. The GTT levels were not considered by the sponsor because the detection was close to the detection limits.

Urinalysis
There were no values outside a normal range , although the osmolarity of the MD group was significantly decreased as compared to control values. No glucosuria was detected in any dog.

Organ Weights + Relative to bodyweight:
There were no organ weights beyond the normal range and the relative organ weights did not show any statistically significant group difference. However, a dog (#10) in the HD group with elevated ALT levels also had a relative liver weight that represented 5.4% of the body weight, greater than any other dog in the study, 4.4% was the most any control group animals. The histopathology of the liver of dog #10 had necrotising inflammation and a moderate degree of eosinophilic inclusions, a gradation of inclusions that was higher than any other dog in the study. In dog #10, the 250 µg/kg/day of dexmedetomidine was hepatotoxic.

Gross pathology

The only macroscopic pathology that was attributed to dexmedetomidine treatment was a pale appearence at the injection site in 3/3 HD animals.

Histopathology

The following are incidences of histopathology that are more frequent in the dexmedetomidine treated animals, however there were no statistically significant differences between treatment groups.

Histopathology	control	dexmedetomidine 10μg/kg/day	dexmedetomidine 50μg/kg/day	dexmedetomidine 250μg/kg/day
Heart - pericardisal adhesions	0/3	0/3	0/3	1/3
Adrenal macrophage infiltrate	0/3	0/3	1/3	1/3
Liver - eosinophilic intracytoplasmic +++	0/3	0/3	0/3	1/3

+++ moderate, the maximum severity observed

Toxicology Summary:

The daily intramuscular administration of dexmedetomidine at doses of 0, 10, 50 and 250 μg/kg/day, in 3 female dogs per dose group, was studied for 28 days. No mortality was observed. A slight sedation was noticed in the LD group for 2-3 hours after injection, the sedation had a duration of 3-4 hours in the MD group and 5-7 hours in the HD group. The mean group body weights were slightly, insignificantly, reduced in the HD group only and one dog in this group did have reduced food consumption.

Opthalmoscopic examinations revealed corneal opacities in 2/3 of the high dose dogs and this was attributed to the decreased blinking and tear formation, pharmacological actions of dexmedetomidine. No significant toxicological changes were observed in either the hematologic or urinalysis parameters. Serum ALT was elevated, 3 to 6-fold, in 2/3 of the HD dogs and one thesc dogs had both the largest relative liver weight in the study and the most severe rating of hepatic eosinophilic intracytoplamic inclusions. These inclusion were observed in 2/3 of the MD group and all 3 in the HD group.

The reviewer agrees with the sponsor the NOAEL was the LD, 10 μg/kg/day, but the ratio with MRHID is less than 1.0. The LOAEL was 50 μg/kg/day and a comparison of the AUC values of 25.7 ng.hr/ml with the human iv 24 hour infusion AUC of 37.76 ng.hr/ml, the human exposure remains greater.

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NDA 21-038

[53]

Study Title: Fourteen Day Intrathecal Toxicity Study of Dexmedetomidine in the Rat (#222)

Study No: 94-019
Vol #37, and page #001:
Conducting laboratory and location: [redacted]
Date of study initiation: November 15, 1994
GLP compliance: No pg 16; QA- Report Yes () No (X)
Methods:
- species/strain: rats/Sprague Dawley outbred
- #/sex/group: 10 males/group
- weight: 210g on arrival, 237-297g during surgery
- dosage groups in administered units: 0, 1.5, 6, 24 µg/10µl (per rat volume)
- route, form, volume, and infusion rate: intrathecal administration, bolus administration in 10 µl volumes
- a fifth treatment group received agmatine, 100 mg/ml solution and 1mg/10µl. However, this was very toxic, causing convulsion and death and is not considered or discussed in the remainder of this review.

Drug, lot#, radiolabel, and % purity: batch 92E245 (pg 17)
Formulation/vehicle: dexmedetomidine hydrochloride in normal saline
Observations and times:
Clinical signs: recorded 3X daily, before dosing, after dosing and late afternoon.
Body weights: weekly
Food consumption and Ophthalmoscopy: none
EKG: Hematology, Clinical chemistry, Urinalysis, Organ weights: none
Gross pathology: brain, lung, spinal cord (cervical, thorasic and lumbar)
Histopathology: brain, lung, spinal cord (cervical, thorasic and lumbar)

Results:
The sample size, 10 per dose, was reduced by catheter disconnection. The final group size for 0, 1.5, 6 and 24 µg/rat/day were 5, 6, 7 and 8, respectively. The injection solutions were analyzed and the concentration of dexmedetomidine was from [redacted]

Clinical signs: Sedation post administration was slight and transient in the LD and intermediate and transient in MD and HD. Piloerection observed in 2/8 of the MD group during weeks 1 or 2.

Body weights
The body weight gains were not effected by dexmedetomidine according to the sponsor, but no tables of mean weight gain per group were available, only individual animal data. Mean weight gain for the 5 control rats was 38.2 g and for the HD group of 8 rats the mean weight gain was

24.9g.

Gross pathology
The macroscopic pathology revealed no drug induced changes, although abnormalities were observed: discharge for eyes, catheter insertion site hemorrhages and nodules, nasal discharge in control rats and areas of alopecia.

Histopathology
The sponsor stated that there were no drug induced histopathology and the group listing (Appendix 4, pg 5-16-34) indicate that the saline controls had as many or more pathological occurances as the dexmedetomidine animals. These pathological findings included focal fibrosis in subarachnoid space and mononuclear inflammatory cells in cervical, thorasic and lumbar spinal cord sections. The inflammatory process also include the brain sections in the controls and LD groups.

The histopathology of the animals sacrificed early due to disconnected catheters was similar to the terminal findings above with no indication of drug induced pathology.

Summary
There were no observed pathological changes by any dose of dexmedetomidine, with the doses of 1.5, 6 and 24µg/10µl/rat/day administered as an intrathecal bolus daily for 2 weeks to rats. The LD produced a slight transient sedation and this was moderate and transient in the MD and HD animals. The were no apparent drug-induced effects on body weight, gross or microscopic pathology, including neurotoxicity, at any dose.

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[54]

Study Title: A Pilot Intrathecal Injection Study of Dexmedetomidine Hydrochloride (Abbott-84599 Hydrochloride) in the Beagle Dog. (#223)
Study No: TB95-216
Vol #37, and page #115 (5-516-115)
Conducting laboratory and location: _____
Date of study initiation: August 14, 1995
GLP compliance: Yes, pg 121
QA- Report Yes, pg 138

Methods: In this pilot study, one male and one female beagle dogs were given daily bolus intrathecal injections of dexmedetomidine. The catheter was inserted at L5 and lumbosacral interface. The catheter was passed subcutaneously to an interscapular exteriorization site.

NDA 21-038

Dosing:
The dose progressed 3, 5, 8, 12, 18, 36, 72 and 144 µg/dog. The male dog subsequently received a dose of 10µg and the female 20 µg. An extra, untreated male received a dose of 40µg. The dose volume was 0.5ml except for the 144µg dose which was administered in 1 ml and the catheter was flushed with 1.5 ml of sterile saline.
- species/strain: canine / beagle
- #/sex/group: 1 / sex (+ 1 spare male for 1 dose)

Drug: dexmedetomidine hydrochloride. Lot 06-038VH [redacted]
Formulation/vehicle: prepared in sterile saline
Observations and times:
Clinical signs: twice a day or more as warranted
Body weights: 9.9kg ♂, 7.3kg ♀
EKG: pre-dosing and 1 and 24hr post-dosing
Respiratory rate: 1 and 24hrs post-dosing after doses >40µg
Hematology, Clinical chemistry, Urinalysis, Organ weights, Gross Pathology and Histopathology were not performed.
Results:
Clinical signs:
Slightly sedated, but standing, ♀ for about 15 minutes, starting at about one-hour post-dosing. After 144 µg, sedation was greater in ♀ and this dog rested in quiet repose, unarousable by voice or paw pinch stimulation, from 20 to 50 minutes post-dosing. After about 1 hour, the female tried to stand and eat. The male at 144µg showed quiet repose and was always arousable. The standing was with difficulty 1.75 hrs post dosing. The male with the 40µg dose had transient hindlimb weakness, but no other signs of sedation.
Body weights; no remarkable change
Electrocardiography: The female had marked decrease in heart rate after 72 µg and both male and female had decreased heart rates after the 144 µg dose. Heart rates were all normal after 24 hours.
Respiratory rate: After the 144 µg dose, both male and female had decreased respiratory rates after one hour and this was normal by 24 hours post-administration.

Summary

This pilot study demonstrated that intrathecal administration of 40µg doses produced transient hindlimb weakness and 72µg produced transient sedation and decreased heart rate in ½ dogs. The dose of 144µg produced moderate to heavy sedation and decreased heart rate and respiration rate in 2/2 dogs. The heavy sedation in the female prevented arousal by voice or paw pinch from 20 to 50 minutes post-dosing.

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NDA 21-038

[55]

Study Title: A 28-Day Intrathecal Injection Toxicity Study of Dexmedetomidine Chloride (Abbott 85499 Hydrochloride) through in the Beagle Dog with a 14-Day Recovery Period. (#224)

Study No: TB95-224/Project #54322
Vol #37 and page # 187 through Vol#38 page 305:
Conducting laboratory and location:
GLP compliance: yes (Vol 37/pg206)
QA- Report Yes (x)(Vol 37/ pg 233)
Methods: Daily intrathecal bolus injections of 0.5 ml of dexmedetomidine hydrochloride, at doses of 0, 2, 12, and 80µg/dog, followed by 1.5 ml of sterile saline, were made for 28 consecutive days.

Dosing:
- species/strain: canine/beagle
- #/sex/group: 3/sex/group
- age: 5 to 6 months at start
- weight: 8-10.2 kg males; 6.4-8.6 kg females
- satellite groups used for recovery: 2/sex in controls and 80 µg salt/day for 14 days
- dosage groups in administered units: 0, 2, 12 and 80 µg/dog/day
- route, form, volume, and infusion rate: intrathecal administration through indwelling catheter, 0.5 ml bolus administration, solutions in normal saline.

Drug, lot# and % purity: dexmedetomidine hydrochloride, lot #06-038VH,

Formulation/vehicle: dexmedetomidine dissolved in sterile saline, 4, 24 and 160 µg/ml. The low dose solution was found to be only 80.5% of the nominal concentration, but the sponsor states that this would not have diminished the appearance of pharmacological effects. Since this dose was without obvious effects, this statement is in question (Vol 37/pg 194).

Observations and times:
Clinical signs: twice daily
Body weights: weighed weekly
Food consumption: daily measured amount presented
Ophthalmoscopy: predosing and during week 4 and week 6 in recovery animals
EKG: predosing, 30 and 60 minutes post first dose and during week 4, 6
Hematology: pretreatment, week 4 and week 6 for recovery animals
Clinical chemistry: pretreatment, week 4 and week 6 for recovery animals
Urinalysis: pretreatment, week 4 and week 6 for recovery animals
Organ weights, Gross pathology, Histopathology: at necropsy
Toxicokinetics: peak plasma levels, 30 minutes post administration, determined Days 1 and 22

Histopathology: at necropsy, following retained, + = examined histopathologically:
All gross lesions, brain+ (including areas of cerebral cortex, thalamic nuclei, mid-brain, medulla and cerebellum), spinal cord +(cervical, thoracic and lumbar regions)*, pituitary, eyes, submandibular salivary gland, heart, thymus. thyroids including parathyroids, lung+ 2 lobes, trachea, esophagus. stomach (body and antrum), duodenum; jejunum, ileum, caecum, colon rectum, adrenals, pancreas, liver two lobes+, gall bladder, kidneys, urinary bladder, aorta (arch and abdominal), uterus, ovaries, spleen, lymph node (submandibular and mesenteric), sternum. femur (with marrow), sciatic nerve+, mammary gland, skin, tongue, skeletal muscle; catheter tip and 1 cm rostral and caudal+, *meninges included the dura mater

Results:
Clinical signs:
At the 2 µg dose, no drug induced changes were observed however, at 12 and 80 µg doses, there was dose-related transient increased incoordination, sedation and eye vascular dialation. There was decreased responding to toe pinch (tested Days 19 and 22 or 23) in two HD dogs, one on Day 19 and 23, and one on Day 22. A third dog displayed a loss of withdrawal reaction 30 minutes post on Day 29 but this was attributed to meningitis.

Body weights:
No treatment related changes were observed in body weights or food consumption.

Ophthalmoscopy:
After 28 days of dosing at 80µg, the reflectivity of the retina and superior tapetum was reduced in most dogs in this HD group, but in none of the MD or control group dogs. The reduced reflectivity was attributed to very mild edema in the retinas and tapeta and was not present at the end of the 14 day recovery.

Electrocardiography:
There was a marked decrease in heart rate 30 and 60 minutes post-dosing on the first day in the HD group, 8/10 dogs. A slight increase in QT interval in 6 of these 8 dogs and one had a second degree atrioventricular block. The QT and AV block were not classified as cardiotoxic events by the veterinary cardiologist (Vol 37/pg 230). Systolic blood pressure and heart rate decreased in the HD male dogs 60 minutes post-dosing and in the HD females, 30 and 60 minutes post the initial dose, Day 1. Heart rate, respiratory rate and systolic blood pressure were unchanged in all treatment groups after 28 days of dosing. The systolic blood pressure was measured indirectly and there were no statistically significant group differences during the entire study. The respiratory rate decreases Day 1, 60 minutes post, was significant in HD group for males and females.

Hematology, Clinical chemistry, Urinalysis, Organ Weights: No drug related changes observed.

Gross pathology:
Animals in both control and dexmedetomidine groups had swelling or thickening of the subcutaneous tissue at the site of surgury and this was considered due to procedures, not drug treatment. A HD male, #4052, had dark foci in the brain and spinal tissue and pale opaque fluid in the cranial cavity.

Histopathology:
The HD male, #4052, had acute meningitis with neutrophil infiltration and hemorrhages in sections of the brain and spinal cord. Two other dogs had slight meningitis, another HD, #4542, and a control #1511.

The catheters were patent in 31of 32 dogs but the placement was found at necropsy (Vol 37/pg 227) to be epidural in 21 dogs, subdural in only 9 dogs and subcutaneous in one dog (HD). This does not agree completely with the tables (Vol. 37/ pgs 234-235), which have 5 catheters outside the meninges at necropsy and 26 inside the meninges, including one inside the spinal cord.

Toxicokinetics: (Vol 37/pg 196)(Vol 38/pg 251, 259) The graphs did not enumerate the number of dogs per dose group, but the tables of individual valuse (V.38/pgs 266-273) indicated that the controls, LD, MD and HD, were 6/sex, 3/sex, 3/sex and 6/sex, respectively. One HD male was classified an "outlier" and removed from analysis and this dog was classified as having a catheter which terminated subcutaneously. The remaining dogs were evidently included in analysis regardless of catheter placement, epidural or subdural.

Pharmacokinetic Evaluation of Abbott-85499 in Plasma 30 minutes Post Administration

Dose Group (µg salt/day)	C_{MAX} (pg/ml)	AUC(0-inf) *(pg*hr/ml)*	$t_{½}$ (hr)
Day 1			
Males			
2	**7.9 + 7.5**	**3.7 + 3.2**	*
12	**89.8 + 49.0**	**103.6 + 39.5**	**0.57**
80	**927.9 + 316.6**	**1610.2 + 264.6**	**0.85**
Females			
2	**9.2 + 8.2**	**10.4 + 9.2**	*
12	**89.2 + 24.8**	**144.9 + 40.2**	**0.77**
80	**1207.8 + 593.5**	**1986.8 + 643.1**	**0.86**
Day 22			
Males			
2	**11.4 + 1.0**	**5.2 + 1.8**	*
12	**80.9 + 21.5**	**114.9 + 17.1**	**0.67**
80	**945.7 + 401.6**	**1658.7 + 602.9**	**0.82**
Females			
2	**14.0 + 1.6**	**12.5 + 4.7**	*
12	**81.6 + 16.0**	**129.4 + 25.2**	**0.61**
80	**1429.5 + 482.2**	**2068.4 + 312.0**	**0.78**

* Unable to estimate apparent plasma elimination half life

The AUC's and C_{MAX} values were non-linear and greater than the dose-proportional relative to the high dose of 80 μg/day. The 12 μg dose was roughly proportional to the 2 μg dose. This was true both on Day 1 and Day 22. The elimination half-life was from 30 to 50 minutes at the two higher dose levels and not estimatable at the low dose.

Summary

Bolus intrathecal administration of dexmedetomidine at 2 μg/day did not produce any observable changes. Slight sedation was observed in two animals at the 12 μg/day dose and all dogs at the 80 μg/day dose and incidence of sedation decreased with duration of the study. This may have been complicated by the catheter placement changing from intrathecal at the start to epidural upon necropsy in 21 of the 32 dogs. The data from the 11 dogs with patent catheters is presented. The epidural doses were probably less sedative. A dose related increased dilation in eye vasculature was seen at 12 and 80 μg/day animals. Incoordination was observed in 2 dogs in the MD group and all dogs in the HD group

An analgesic test (Vol 38 /pg 196) of toe pinch was reported to have been done with all dogs on Days 19, 22 and 23 and 2 HD animals were reported to show lack of withdrawal 15 minutes post-dosing. However, no tables or notations were in the report. The heart rates and respiration rates were decreased in the HD group 30 and 60 minutes post administration on the first Day but not on Day 28 and the prolonged QT interval was not considered indicative of cardiotoxicity by veterinary cardiopathologist.

No clinical chemistry, hematology or urinalysis parameter changes were related to drug administration and the histopathology observations were related to invasive actions common to intrathecal catheterization and no drug specific effects were seen .

The lack observable neural or organ toxicity suggests that the top dose, 80μg, was an NOAEL dose although the dogs in this group displayed the pharmacological effecsts of sedation, ataxia, analgesia and increased vasodialation in the eye vasculature. The TK data estimated NOAEL Cmax concentrations to be from 0.95 ng/ml in males to 1.43 ng/ml in females and AUC values of 1.66 ng.hr/ml to 2.07 ng.hr/ml in males and females respectively.

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