

4.4 Biodistribution Studies

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4.4 Biodistribution Studies

Overview

Several studies were completed to determine the PK and biodistribution of OP-1 following various routes of administration in several species. Rats were used for most studies, but monkeys were used for comparison in one study. Rabbits were also used for a spinal fusion/PK study because they are useful for the simultaneous measurement of efficacy and distribution. In general, OP-1 was not widely distributed in the body after administration. After IV administration, its half-life was only about 0.3 to 1.5 hours in rats and monkeys. When administered as OP-1[®] Putty in spinal fusion models, blood levels were persistent but remained low and never exceeded more than 3% of the total administered dose. OP-1 did not accumulate to any significant extent in any major organs except the thyroid (representing free iodine as expected for a protein radiolabeled with iodine) and at the surgical sites. OP-1 was mainly cleared by the kidneys. The PK results for OP-1 are consistent with the profile of a device whose effects are limited to the site (or compartment) of administration.

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Table 4-2: Tabulated Summary of GLP Pharmacokinetic Studies of OP-1 Given IV or by Implantation

Stryker Biotech Study ID	Species	Test Article/ Lot No.	Dose (mg/kg)	Cmax (ng/mL)	Tmax (h)	T1/2elim (h)	AUC (ng.h/mL)	Kel (hrs-1)	Cl (mL/min/kg)	Vd (L/kg)	Key Findings
00-038 (Refer to P060021/A011 Appendix 1a-32, Section 2.5, page 30)	Rat	OP-1 in 20mM acetate buffer; Bulk Lots AA5J003 (37A) and AA7J002 (37B)	0.025 (37B)	98.1	0.096	0.827	58.07		8.099	0.514	Rapid biphasic elimination at all doses. AUC proportionate w/dose & PK parameters linear. Cl similar to glomerular filtration rate suggests predominant renal Cl. Vd equivalent to total body water suggests minimal tissue penetration.
			0.25 (37B)	1293	0.086	1.053	746.1	-	5.962	0.526	
			2.5 (37B)	9200	0.096	1.426	8740	-	5.025	0.618	
			3.5 (37B)	8896	0.163	1.342	10081	-	5.975	0.673	
			0.25 (37A)	1288	0.077	1.636	849.1	-	5.025	0.704	
00-002	Monkey	OP-1 in 20mM acetate buffer; Bulk Lot AA7J002	0.025	188	0.100	0.328	97.55	-	4.152	0.118	Rapid biphasic elimination at 2.5mg/kg. AUC proportionate w/dose & PK parameters linear. Cl similar to glomerular filtration rate suggests predominant renal Cl. Vd equivalent to total body water suggests minimal tissue penetration.
			0.25	944	0.083	0.519	476.0	-	9.855	0.355	
			2.5	14133	0.083	1.417	10515	-	4.055	0.487	
97-024	Rat	OP-1 in 20mM acetate buffer; Bulk Lot AA5J003	0.035	83a	.08	8.0	539.8	0.086	-	-	Dose independent linear kinetics observed over dose range. Majority sequestered at early time points by liver. Transient accumulations of radioactivity in liver, skin, lung, spleen, testis, & thyroid. Significant levels observed by autoradiography in bladder, tooth root & stomach, & small intestine at early time points
			0.35	773a	.025	8.5	6193	0.082	-	-	
			3.5	6500a	.08	7.5	47620	0.093	-	-	
04-008	Rabbit	OP-1 Putty; Lot AH03B102	0.54	239	24	-	26400b	-	-	-	Radioactivity in circulation low: ≤2% radioactivity dose. Surgical site retained most of radioactivity. Little accumulation in other areas/tissues. Radioactivity excretion mainly via urine. Fusion rate 100% in 35-day group.
07-012 (Refer to P060021/A011 Appendix 1a-1)	Rabbit	OP-1 Putty (OP-1 bulk added to collagen/CMC) Lot AA05J110.2)	.75	16	420	-	8015	-	-	-	Release of OP-1 from collagen/CMC carrier was slow and not dose dependant. ALL animals treated with OP-1 Putty, regardless of dose, went on to fused by day 35.
			1.5	17	840	-	7817	-	-	-	
			3.0	19	638	-	8748	-	-	-	

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Results

Intravenous Studies

Cynomolgus monkeys were given a single IV bolus of OP-1 at 0.025, 0.25 and 2.5 mg/kg for the purpose of defining its PK profile after systemic administration in a nonhuman primate (Stryker Biotech Study 00-002; P060021, Appendix 2, page 1428). An ELISA assay was used to assess OP-1 levels with a lower limit of quantification (LLOQ) of 7.9 to 8.8 ng of OP-1. Exposure was dose proportional and clearance was rapid with a biphasic elimination profile observed at the high-dose level (2.5 mg/kg). Elimination half lives were 0.328, 0.519, and 1.417 hours for the respective doses. Clearance was in the range of 4.2 to 9.9 mL/min/kg and was similar to the glomerular filtration rate, indicating that renal (rather than hepatic) clearance may have been predominant. The apparent volume of distribution (Vd) was low (Vd in the range 0.12 to 0.49 L/kg). This volume was consistent with the total body water for the monkey indicating that OP-1 was not distributed into a deep compartment in tissues.

Rats were given a single IV bolus dose of OP-1 at 0.025, 0.25, 2.5, and 3.5 mg/kg for the purpose of defining the PK profile after systemic administration and to compare the results with those obtained in a nonhuman primate (Stryker Biotech Study 00-038; P060021, Appendix 2, page 1280). As in the monkey studies, the levels of OP-1 were measured by ELISA assay with a LLOQ of 2.5 ng/mL. Exposure was dose proportional and clearance (Cl) was rapid with a biphasic elimination profile observed for all dose levels. Elimination half lives were 0.827, 1.053, 1.426, and 1.342 hours for the four respective doses of OP-1. Cl was in the range of 5.0 to 8.1 mL/min/kg and was similar to the glomerular filtration rate in the rat, indicating that renal (rather than hepatic) Cl may have been predominant. The Vd was low (Vd in the range 0.51 to 0.70 L/kg). This volume was consistent with the total body water for the monkey indicating that OP-1 was not distributed into a deep compartment in tissues. No differences were observed between the PK parameters of OP-1 following the administration of 0.25 mg/kg of protein produced by two different manufacturing processes (process 37B or 37A). Moreover, PK characteristics for the rat and monkey were similar for all measured parameters at the doses used.

A study using radiolabeled OP-1 (Stryker Biotech Study 97-024; P060021, Appendix 2, page 1551) was conducted to determine the rate of Cl from the systemic circulation in rats following IV administration. Iodinated OP-1 was used in doses ranging from 0.035

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to 3.5 mg/kg and half-life was determined by measuring ^{125}I in whole blood. The elimination of radioactivity from the blood stream exhibited first order kinetics ($k = 0.82$ to 0.93 h^{-1}). The terminal half-life of total and TCA-precipitable (protein bound) radioactivity was independent of dose over the dose range in both male and female rats. Thus, the mean terminal half-life of total radioactivity was 8.0 and 5.9 hours in male and female rats, respectively. The mean terminal half life of protein bound radioactivity was estimated to be 15.1 and 9.6 hours, respectively. Kinetics appeared to be linear and dose-independent over the dose range 0.035 to 3.5 mg/kg. Peak concentrations in the blood, liver, lungs, spleen, and thyroid were much higher than those in other tissues regardless of the sex of the animal. Of these tissues, the liver contained more than 50% of the dose at 0.08 and 0.25 hours, declining to 1% or less at 24 hours. It appears that the liver rapidly removes the ^{125}I -OP-1 from the blood because the blood contains no more than 27% of the radioactivity at the early time points. Brain, heart, lymph nodes, pancreas, ovary, testis, thymus, and pituitary gland generally contained less than 1% of the dose of radioactivity at each time point. Radioactivity declines to a minimum in all tissues except the thyroid at 24 hours.

Implant Studies–Spinal Fusion

A study was done to determine the disposition of OP-1 in male New Zealand white rabbits following single administration of ^{125}I OP-1[®] Putty into a spinal fusion surgical site (Stryker Biotech Study 04-008; P060021, Appendix 2, page 1664). The radioactivity was measured in blood plasma and tissues over a 35 day observation period. OP-1[®] Putty was administered into the fusion site at a dose level of 0.54 mg/kg (49 μCi /animal). Blood samples were collected from animals at selected time points prior to euthanasia. Following blood collection, the animals were euthanized and radioactive concentration determined in tissues by quantitative whole-body autoradioluminography. Urine, feces, and cage washings were collected from animals over designated intervals up to 35 days post-dose. The radioactivity in these samples and in blood/plasma samples was measured. Three (3) animals were euthanized 35 days following administration and processed for histopathology evaluation.

Following administration of ^{125}I OP-1[®] Putty at the fusion surgical site, the observed radioactivity concentrations in blood were low, ranging from 0 to 0.239 $\mu\text{g eq/mL}$ with the extrapolated percent of dose below 3% at each time point. In whole blood, maximum blood levels were achieved at about 24 hours. An AUC of 26,400 ng eq h/mL was determined based on measurements up to the last time point. The $t_{1/2}$, AUC_{0-inf}, and

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kh-1 could not be estimated due to an inability to characterize the terminal phase. For plasma, the $t_{1/2}$ was 500h, the AUC_{0-inf} was 45,300 ng eq.h/mL, and the kh-1 was 0.00139. Excretion of radioactivity was mainly via the urine giving a total mean recovery of 104 to 111% over 35 days. Only about 5% of the radioactivity was found in the feces and less than 2.5% was found in cage washes.

The highest tissue radioactivity concentration and AUC values were obtained from the surgical sites, urinary bladder wall, urinary bladder contents, and thyroid gland (thyroid levels were attributed to free ¹²⁵I). These were the only tissues/areas with quantifiable concentrations of radioactivity 14 days following administration. Limited exposure was observed in the adipose tissue (white and brown fat), brain, eye, pituitary gland, skeletal muscle (dorsal), and spinal cord where the mean radioactivity concentrations were below the limit of quantification (LOQ) for all time points.

A semi-quantitative assessment of spinal fusion was done after a single administration of ¹²⁵I-OP-1[®] Putty to insure that the protein was biologically active. Unilateral or bilateral fusion was usually observed in the animals assessed 35 days after surgery. Successful arthrodesis was also evident, as evaluated by manual palpation, plain radiographs, and undecalcified histopathological evaluation.

A second PK study using OP-1 putty implantation was performed with the primary intent of studying the toxicity of the putty combined with various concentrations of OP-1 (Stryker Biotech Study 07-012; P060021/A011, Appendix 1a-1). Five treatment groups (N = 3/gender/group), included a sham surgery (untreated) control group (Group 1) and four treatment groups. Animals in Groups 1 through 4 had surgery. Group 1 (sham) had surgery alone (0 mg/kg OP-1) and no placement of material. Groups 2 through 4 had 3 mL of OP-1 Putty implanted; 1.5 mL was placed on each side of the vertebrae between transverse processes L4 and L5. The concentrations of OP-1 in Groups 2, 3 and 4 were 1, 2 and 4 mg/mL, respectively. Animals, assigned to Group 5, were injected IV with 1 mg/kg of 6 mg/mL OP-1 on Day 0; these animals served as positive controls for the serum OP-1 ELISA only. TK serum samples were also collected at various time points.

The animals implanted with 3 mL of 1, 2, or 4 mg/mL OP-1 Putty had low serum concentrations of OP-1 (10 to 50 ng/mL range) on Days 14 and 35. Males in Groups 2 (1 mg/mL OP-1) and 3 (2 mg/mL OP-1) had higher AUCs compared with the females; females in Group 4 (4 mg/mL OP-1) had a higher AUC compared with the males. Because all of the animals implanted with OP-1 Putty had vertebral fusion with bone

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growth, the biologic significance of the gender differences is not clinically meaningful or toxicologically relevant. Animals injected IV once with 1 mg/kg OP-1 had serum OP-1 detectable for a short time (minutes to 2 hours) in the 0 to 100 ng/mL range.

Pharmacokinetics and Biodistribution Conclusions

OP-1[®] Putty had nearly ideal PK characteristics for a device intended for local use. Systemic PK studies demonstrated that OP-1 is rapidly cleared from the blood after systemic injection. Its PK characteristics were very similar in nonhuman primates and rats. In addition, there were no significant differences in PK based on the sex of the animals (in rats). The clearance rate of OP-1 approximates the glomerular filtration rate, indicating it is cleared by the kidneys. Moreover, its volume of distribution was similar to the volume of whole body water suggesting that OP-1 was not distributed into the deep tissue compartment. Although low serum levels of OP-1 were observed after implantation, OP-1 did not accumulate in any tissue for a long period of time except for the desired persistence at the surgical site in rabbits. The relative lack of exposure away from the surgical site may have contributed to the lack of adverse systemic effects observed in toxicology studies.