

**OVERALL SUMMARY AND EVALUATION OF
PRECLINICAL PHARMACODYNAMICS, TOXICOKINETICS AND TOXICOLOGY**

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Pharmacodynamics

Bosentan is a competitive inhibitor of endothelin receptors. *In vitro*, bosentan potently inhibits binding of endothelin-1 to human ET-A and ET-B receptors. Potency on ET-A receptors was only slightly greater than potency on ET-B receptors. Functionally, bosentan inhibits contractile effects of endothelin-1 in rat aorta and human blood internal mammary artery and vein. Oral administration of bosentan to rats inhibits pressor effects of big ET-1, which is the precursor to ET-1. Although bosentan decreases systemic blood pressure in rat models of hypertension, it does not lower blood pressure in normotensive rats and dogs at doses that block pressor effects of exogenous endothelin. Administration of bosentan increases plasma levels of endothelin; the consequence of this increase is not clear.

The sponsor provided preclinical evidence supporting a therapeutic role of bosentan in pulmonary hypertension. Oral administration of bosentan¹ decreased pulmonary arterial pressure in rat models of pulmonary hypertension. The predictive power of these preclinical assays for human therapeutic activity is not known.

Toxicokinetics

Plasma exposures to bosentan and its metabolites were monitored in dogs, rats and mice. Similar to humans, bosentan metabolites comprised a small percentage of drug-related product found in plasma. Although not all metabolites were found in all species evaluated, dogs exhibited all three human metabolites, and Ro 48-5033, a major human metabolite, was found in all three species.

Exposure to bosentan decreased with repeated administration in rats and dogs, similar to findings in humans. This decrease in exposure is likely due to induction of hepatic metabolism. In dogs, bosentan metabolism was 5-fold more rapid in hepatocytes from the livers of dogs given bosentan for 12 months than in hepatocytes from livers of dogs given vehicle over this same time period.

In chronic toxicology studies, dogs given bosentan orally at doses up to 50 times the human therapeutic dose exhibited higher systemic plasma exposures to unchanged parent than rats given bosentan orally at doses up to 100 times the human therapeutic dose (2-year carcinogenicity study) and mice given bosentan orally at doses up to 75 times the human therapeutic dose (2-year carcinogenicity study). Exposure in males and females were similar in dogs, but female rats and mice showed higher exposures than male rats and mice. Animal exposures cannot be compared to exposure in pulmonary hypertensive patients since exposure in these patients is unknown. Plasma protein binding of bosentan in dogs, rats, mice and rabbits was high and similar to that seen in humans.

Species	Maximum Oral Dose Evaluated		Study Duration	Sex	Bosentan AUC (µg.h/ml)
	(mg/kg/day)	Multiple of Human Therapeutic Dose*			
Dog	500	50	12 month	Male	466
				Female	510
Rat	3000	100	24 month	Male	119
				Female	255
Mouse	4500	75	24 month	Male	30
				Female	55

* Human therapeutic dose of 125 mg, bid; animal doses were corrected for body surface area

¹ 100 mg/kg/day, 3 times recommended human therapeutic dose.

General Toxicology

Hepatic findings

Bosentan affected the liver in the 12-month oral toxicity study in dogs², with elevated mean serum alkaline phosphatase (ALP) levels seen in male and female dogs given higher doses. In one of four male dogs given the highest dose,³ marked increases (25-30 fold) in ALP, alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) and glutamate dehydrogenase (GLD) were observed, along with a 2-3 fold elevation of aspartate aminotransferase (AST). Clinical signs were not observed at the time of maximum liver enzyme elevations. Elevated transaminases were preceded by small (2-fold) increases in ALP at earlier time points.

Clinical signs were noted in this high dose animal approximately 2 months after transaminases were observed to be markedly elevated.⁴ Although liver transaminases were elevated on the day of clinical signs, increases were moderate, and bilirubin was not increased. Clinical signs appeared to be test agent related, since upon cessation of treatment with bosentan, this dog recovered, and when rechallenged, clinical signs reappeared; liver enzymes were not monitored when clinical signs reoccurred. Clinical signs did not reappear after bosentan administration was terminated permanently. At sacrifice 38 days after treatment termination, serum enzymes were only slightly elevated in this dog compared to baseline, and there was no evidence of hepatic necrosis or fibrosis. Consequently, liver effects appeared reversible.

The pattern of liver enzyme increases seen with bosentan suggests primary biliary obstruction with secondary hepatocyte necrosis, or cholestatic hepatotoxicity, since increases in ALP preceded increases in ALT, AST and GLD. Increases in ALP and GGT are indicative of biliary injury, and are not elevated in cases of primary hepatocyte necrosis. Consistent with this interpretation is pigmentation of bile duct canaliculi and gallbladders observed histopathologically in dogs given the highest dose. Additionally, biliary secretion appeared to be inhibited by bosentan, since biliary cholesterol was reduced by higher doses (18 and 50 times the recommended human dose), and biliary phospholipids and bile acids were reduced. Finally, non-cholesterol (dark, friable) gallstones were observed in one of four female dogs given 18 times the recommended human dose, suggesting the possibility of external biliary obstruction. The lack of bile duct proliferation in dogs given the highest dose indicate that chronic biliary obstruction did not occur in this study.

Liver effects were also seen in shorter-term studies in dogs and rats.

Species	Adverse Liver Findings with Bosentan	Oral Dose		Study Duration
		mg/kg/day	Multiple of Recommended Human dose*	
Dog	Elevated Alkaline Phosphatase	400	40 X	6 Months
	Periacinar Hypertrophy	400	40 X	
	Liver Hypertrophy	60	5 X	
	Increased ALT, Alkaline Phosphatase and liver wt	1000	100 X	4 Weeks
	Bile duct proliferation, single cell necrosis			
Rat	Increased liver wt	1000	33 X	6 Months

*Recommended human dose is 125 mg, bid.

² Dogs were given 60, 180 or 500 mg/kg/day orally for 12 months (6, 18 and 50 times the recommended human dose).

³ This dog was given 500 mg/kg/day, or 50 times the recommended therapeutic human dose of 125 mg, bid.

⁴ This high dose dog was found recumbent in the kennel 1.5 hours after dosing, was apathetic, sedated and the nictitating membrane was prolapsed. The dog appeared dehydrated with enophthalmia and exsiccosis, delayed capillary refilling and pale mucus membranes. Heart rate was 60 beats per minute, arrhythmic and breathing rate was 20 per minute. An ECG showed sinus bradycardia, which is normal for conscious dogs. Symptoms were absent the following morning, but test agent was discontinued for 3 days.

The sponsor suggests that inhibition of the canalicular bile salt export pump, with resultant accumulation of bile salts, accounts for the adverse liver findings. This is supported by elevated serum bile acids and alteration of bile composition in dogs given bosentan for 12 months.

Anemia observed clinically and preclinically

Bosentan affected erythroid and coagulation parameters in male and female dogs in the 12-month toxicity study. Marked transient reductions in RBCs, hemoglobin and platelets, and increases in fibrinogen and reticulocytes were observed in one of four female dogs given the highest dose (50 times the recommended human dose) coincident with clinical signs of toxicity. This female dog recovered after daily oral dosing with bosentan was temporarily discontinued. A transient 14% decrease in hemoglobin and hematocrit coincident with a marked increase in reticulocytes was also seen in one of four male dogs given the highest dose. Changes in erythroid parameters observed in the 12-month study in dogs are consistent with small decreases in hemoglobin and hematocrit seen previously in 6-month oral toxicity studies in dogs and rats given ≥ 6 times the recommended human dose. Findings in animals are consistent with clinical findings of anemia observed in patients.

The sponsor suggests that decreases in hemoglobin and hematocrit seen in animals and humans are due to a pharmacodynamic effect of bosentan to reduce vascular permeability (since endothelin-1 increases vascular permeability), and therefore increase plasma volume, i.e., a dilutional effect. Although this hypothesis might account for bosentan's effects on hemoglobin and hematocrit, other cellular components did not appear to be decreased along with hemoglobin and hematocrit. Additionally, this hypothesis seems unlikely to account for the marked (75%) and moderate (14%) transient reductions in hemoglobin and hematocrit, and marked increases in reticulocytes observed in high dose dogs in the 12 month oral toxicology study.

Testicular tubular atrophy

In rats given bosentan orally in a 2-year carcinogenicity study, the incidence of testicular tubular atrophy was increased at all doses administered.^{5,6} While this finding was not dose-related, plasma exposures (AUCs) appeared to have plateaued at doses in which testicular atrophy was observed. Additionally, AUCs at the higher doses were only 2-3 fold higher than that seen at the lowest dose given.

Marked testicular tubular atrophy was also observed in one of eight rats given bosentan at 30 times the recommended human dose for 4-weeks (1000 mg/kg/day, mid dose group), while severe testicular tubular atrophy was observed in rats given 6 times the recommended human dose (200 mg/kg/day, mid dose treatment group) for 26 weeks (2 of 2 rats examined). In both the 4- and 26-week studies, testicular tubular atrophy was not observed in concurrent control or high dose groups.

In contrast to findings in rats, the incidence of testicular tubular atrophy was not increased in mice given bosentan orally for two years.⁷ However, AUCs in mice were lower than those observed in rats at doses associated with testicular tubular atrophy. AUCs in mice given the highest dose were 3-fold less than those observed in rats at doses associated with testicular tubular atrophy. The incidence of testicular tubular atrophy was not increased in dogs given bosentan orally for 6- and 12 months. Although AUCs in dogs were considerably higher than those observed in rats in which testicular atrophy was observed, sample sizes in the dog studies are extremely small (4 dogs/sex/dose), and may be insufficient to pick up this effect.

⁵ Rats were given 125, 500, 2000 and 3000 mg/kg/day for two years (4, 16, 66, and 100 times the recommended human dose).

⁶ The incidence of testicular tubular atrophy ranged from 24-30% in rats given bosentan for two years vs 6% in concurrent controls. All severity grades appeared to be increased by bosentan.

⁷ Mice were given 100, 450, 2000 and 4500 mg/kg/day for two years (1.6, 7.5, 33 and 75 times the recommended human dose).

Histopathologically, oligospermia was observed in the testes in one of two dogs given bosentan orally at 100 times the recommended human dose for 4-weeks.⁸ However, sperm counts, sperm motility and fertility of male rats were not affected by bosentan given orally at doses up to 50 times the recommended human dose.⁹ The lack of effect on sperm counts, sperm motility, and fertility of male rats suggest the following possibilities:

1. testicular tubular atrophy is not drug-related,
2. testicular tubular atrophy is not indicative of functional effects on spermatogenesis,
3. testicular tubular atrophy and functional effects on spermatogenesis may require chronic dosing.

Miscellaneous findings observed in the 12 month oral toxicity study in dogs

Renal interstitial fibrosis, consistent with an old infarct, was observed in one of four male dogs and one of four female dogs given 50 times the recommended human dose for 12 months. Moderate bilateral retinal degeneration was seen in one of four male dogs given the same high dose.

Developmental Toxicology

Bosentan was teratogenic and fetotoxic when given orally to pregnant rats during the period of organogenesis at 2, 10 and 50 times the recommended human dose.¹⁰ Agenesis of the palate, craniofacial abnormalities (including shortened, misshapen mandibles, fusion of pterygoid process with tympanic annulus, abnormal zygomatic arch and shortened tongues), anophthalmia, microphthalmia and blood vessel abnormalities (abnormal origin of the right subclavian and innominate arteries) were observed in litters from bosentan-treated dams. Effects were dose-related.

Stillbirths were increased and survival of pups was markedly decreased in a dose-related way in litters from dams given 10 and 50 times the recommended human dose. The duration of gestation was slightly increased (by 1 day). The doses at which adverse developmental effects were not observed were less than 2 times the recommended human dose.

Adverse Developmental Finding	Lowest Dose Observed		No Observed Adverse Effect Level	
	mg/kg/day	Multiple of Recommended Human Dose*	mg/kg/day	Multiple of Recommended Human Dose*
Agenesis of the palate	300	10X	60	2X
Bent hyoid process, misshapen hyoid bone	60	2X	<60	<2X
Misshapen mandibles, abnormal zygomatic arch, shortened tongue, fusion of pterygoid process with tympanic annulus	1500	50X	300	10X
Anophthalmia/microphthalmia	300	10X	60	2X
Abnormal blood vessels	1500	50X	300	10X
Stillbirths	60	2X	<60	<2X
Decreased pup survival	300	10 X	60	2X
Increased gestation duration	300	10X	60	2X

* The recommended human dose is 125 mg, bid.

In utero exposure appears necessary for teratogenesis and lethality, since a litter exchange study showed litters raised by drug-treated dams to survive normally and to show no terata. Although litters of pregnant rabbits given bosentan during the period of organogenesis did not show adverse developmental findings in the absence of maternal toxicity, exposure (AUCs) in rabbits were less than AUCs in rats given teratogenic doses of bosentan. In an *in vitro* assay with mouse palatal explants, bosentan concentration-dependently prevented closure of the palate.

⁸ Dogs were given 1000 mg/kg/day orally for 4-weeks.

⁹ Rats were given 60, 300 and 1500 mg/kg/day for 29-40 days (2, 10 and 50 times the recommended human dose).

¹⁰ Pregnant rats were given 60, 300 and 1500 mg/kg/day orally during the period of organogenesis (2, 10 and 50 times the recommended human dose).

Adverse developmental findings observed with bosentan are likely a class effect of endothelin receptor antagonists, and are likely due to endothelin receptor blockade, since other endothelin receptor antagonists and a knockout model for endothelin shows similar effects.¹¹ Consequently, adverse developmental effects appear probable at bosentan doses required for therapeutic activity.

Genotoxicity

Bosentan was negative for genotoxicity in both *in vitro* and *in vivo* assay systems, as outlined in the ICH guidelines for evaluation of genotoxicity. Bosentan was negative for mutagenicity *in vitro* in the Ames bacterial mutagen assay, the Chinese hamster lung (V79/HPRT) assay and the rat hepatocyte unscheduled DNA repair assay. Bosentan was negative for clastogenicity *in vitro* in human peripheral lymphocytes at concentrations that showed cytotoxicity, and *in vivo* in male mice given single oral doses up to 33 times the recommended human dose.¹²

Carcinogenicity

Rats

In a two-year oral carcinogenicity assay, male and female rats were given bosentan at 4, 16, 66 and 100 times the recommended human dose.¹³ The highest two doses given exceeded the maximum tolerated dose in females since body weights were significantly lower than concurrent control at these doses. In males, exposure plateaued at doses ≥ 16 times the recommended human dose.

CDER's statistical analysis of tumor data shows a significant increase of astrocytomas of the brain in male rats. Astrocytomas were not seen in rats given 4 times the human dose or in concurrent control groups.

Mice

In a two-year oral carcinogenicity assay, male and female mice were given bosentan at 1, 7.5, 30 and 75 times the recommended human dose.¹⁴ The highest doses given were adequate in male and female mice based on saturation of exposure at doses ≥ 30 times the recommended human dose. The results of this study are under consideration.

¹¹ Treinen KA et al., *Teratology*. 59: 51-59 (1999).
Kurihara Y et al., *Nature*. 368: 703-710 (1994).
Kurihara Y et al., *J Clin Invest*. 96: 293-300 (1995).
Lehrer SB et al., *Teratology*. 55: 42 (1997).

¹² Mice were given a single oral dose of 500, 1000 or 2000 mg/kg (4, 16 and 33 times the recommended human dose).

¹³ Rats were given 125, 500, 2000 and 3000 mg/kg/day by dietary administration for 24 months.

¹⁴ Mice were given 100, 450, 2000 and 4500 mg/kg/day by dietary administration for 24 months.