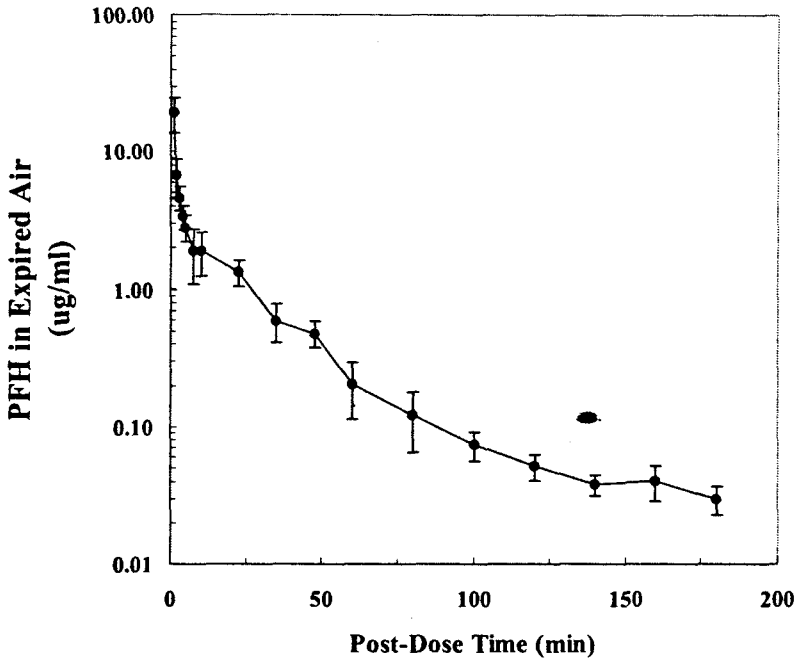

Figure 3. Time-Course of PFH Excretion from Expired Air in Rat after IV Injection of AF0150. The expired air samples were collected with tube sampling methods (referred to Figure 2) in real time after a single bolus IV injection of 20 mg/mg AF0150. Data are mean ± SD of 4 rats (2 each sex).

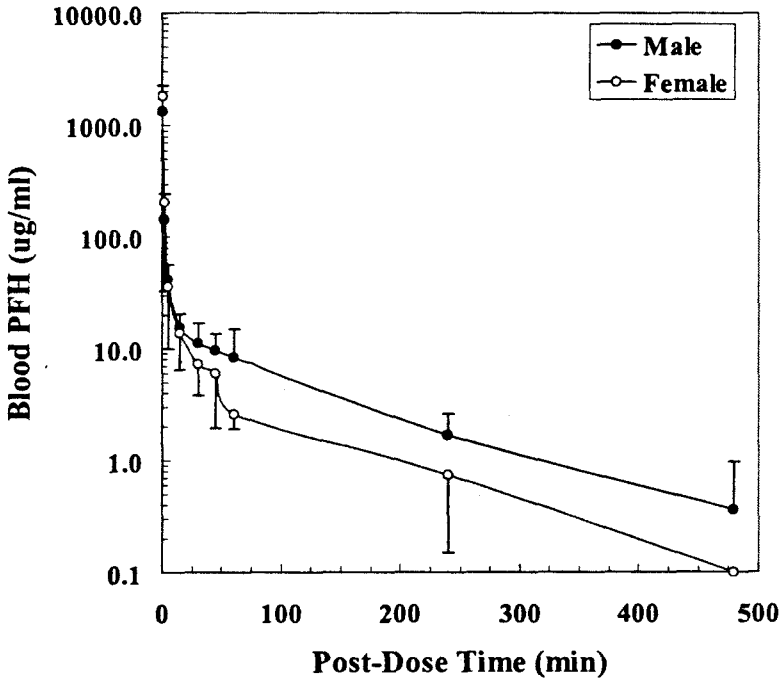

Figure 4. Time-Course of PFH Elimination from Blood in Rat after IV Injection of AF0150. Blood samples were collected from a femoral vein after a single bolus IV injection of 20 mg/mg AF0150. Data are Mean ± SD of 4 rats each sex.

Discussion and Comments

1. Pharmacokinetics of AF0150 following single bolus IV injection at 20 mg/kg (26-fold PCD) in rat was measured based on PFH elimination from expired air and blood. About 90% of the administrated PFH was excreted from lungs within the first 3 hours post dosing and was almost completely eliminated within 48 hours. PFH in blood had the same profile as in expired air, with a two-compartment model. Blood PFH level decreased by 78% during the first 2 minutes post dosing, and was non-detectable by 24 hours.

2. The terminal elimination half-life of blood PFH was about 88 minutes. However, this was based on the pooled data from individual animals. Due to technical difficulties to accurately measure blood PFH level, as noted by the great variations in PFH values, the individual half-life, as well as other PK parameters, need to be calculated and the range of half-life of PFH elimination should be provided.

3. Both individual and summary PK parameters for the expired air PFH study need to be calculated, which could be better to compensate the defect of blood PFH measurement (difficulty in blood sample handling).

4. More than one AF0150 dose need to be tested for pharmacokinetic analysis of PFH in expired air and blood. Also it would be best if the kinetics of microbubbles in blood, both microbubble size and density, would be demonstrated.

5. There was great variation in PFH levels of reconstituted AF0150 (pre-dosing and post-dosing). The relationship between PFH levels and microbubble profile (density and size) was not addressed in this or other pharm/tox studies. The sponsor stated that "these values are typical of our previous experience with the reconstituted product and verifies that accurate and reproducible dose preparation was accomplished". More information is needed to support this.

Summary of Pharmacokinetics/Toxicokinetics Study

Only one pharmacokinetic study was conducted in rats to determine the kinetic profiles of perflexane (PFH, one of the major components in AF0150 microbubbles) in expired air and blood, and to estimate pharmacokinetic parameters of AF0150. The animals received a single bolus intravenous injection of AF0150 at 20 mg/kg (26-fold PCD based on the body surface area) followed by 48-hour observation of PFH in expired air and blood (from femoral vein).

PFH elimination profiles in expired air revealed that about 90% of the administrated PFH was excreted from lungs within the first 3 hours post dosing and almost completely eliminated within 48 hours. PFH in blood had the same profile as in expired air, with a two-compartment model. PFH levels decreased by 78% within the first 2 minutes post dosing, and became non-detectable by 24 hours. The terminal elimination half-life of blood PFH was about 88 minutes based on the pooled data from individual animals.

No distribution and metabolism studies on AF0150 and/or PFH were submitted with this NDA. The sponsor stated that there was no metabolites of PFH in urine and tissues in rat according to a literature report (1). However, in the supporting literature article, PFH was administrated to rats by inhalation (2-hours exposure) instead of intravenous injection. Besides, there was no coadministration of other amphiphilic compounds formulated in AF0150 such as DMPC, HES and Poloxamer-188. The difference in administration route and interaction with other compounds may change the metabolic profile. Identification of PFH forms in expired air and blood could provide valuable information while PFH was quantified with [redacted] during this study.

The fate of the other AF0150 components was not addressed. DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine), HES (m-hydroxyethyl starch) and Poloxamer-188 are other components in the AF0150 formulation, and their pharmacokinetics was not investigated. The sponsor provided some literature and a brief review regarding the pharmacokinetics of DMPC-containing liposomes. In only one clinical study cited by the sponsor, IV injection of 99mTc-lyposome composed of DMPC and DMPG (dimyristoylphosphatidylglycerol) showed clearance of the liposome by macrophages of liver and spleen (2). Based on the literature report, due to nature of DMPC as a phospholipid and its minimal contribution into blood phospholipid pool (400-1000-fold less) after IV administration of 200 mg AF0150, the sponsor concluded that DMPC is safe. However, DMPC is a semisynthetic phospholipid and not natural endogenous lipid, and it is not formulated as a liposome in AF0150. Therefore, knowledge about the pharmacokinetics of DMPC in AF0150 is still important.

Polozamer-188 is a nonionic surfactant with a molecular weight of 8350. It is an approved drug and has been used orally for stool-wetting and stool-softening in the clinical setting. However, the pharmacokinetics and safety via intravenous administration are unknown.

Hydroxyethyl starch (HES) is commonly used for plasma volume expansion, and FDA-approved HES product includes Hetastarch and Pentastarch. Literature reported that HES was taken up by macrophages and parenchymal cells in organs, which frequently resulted in cell vacuolation (3-

6). It was also found that species-dependent elimination of HES and clearance of HES from spleen and liver was slower in rats than in dogs. These may partially explain why vacuolated macrophages were observed in rats but not in dogs following AF0150 administration.

Conclusion and Comments

The major elimination route of PFH, one of AF0150 major components, was through expiratory air. At IV bolus dose of 20 mg/kg (26-fold) in rats, about 90% of PFH was excreted from expired air within 3 hours post dosing. PFH in blood decreased by 78% within the first 2 minutes post dosing, and became non-detectable by 24 hours. The terminal blood half-life of PFH was 88 minutes. Kinetics profiles of PFH in both expired air and blood fitted into a two-compartment model. The following issues need to be further addressed:

1. Due to technical difficulties to accurately measure blood PFH level, as noted by a great variations in blood PFH values, the expired air PFH kinetics need to be individually processed in more detail.

2. More dose groups and another animal species need to be included in pharmacokinetic analysis. The fate of DMPC and Poloxamer-188 following IV administration still needs to be addressed.

3. There was great variation in PFH levels of reconstituted AF0150 (pre-dosing and post-dosing). The relationship between PFH levels and microbubble profile (density and size) was not addressed in this or other pharm/tox studies. The sponsor stated that "these values are typical of our previous experience with the reconstituted product and verifies that accurate and reproducible dose preparation was accomplished". However, the dosage in most pharm/tox studies was verified by osmolality measurement. More information is needed to support this.

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26. SAFETY PHARMACOLOGY

Report Number: EB-95-19-Amended
Hemodynamic and Cellular Responses to Intravenous AF0150 in Anesthetized Rabbits

Report Location:	Vol. 009, p324-334
Report date:	Feb 2, 1996
Study Facility:	
In-life phase:	October 1994 and July/August 1995
GLP Compliance:	No
AF0150 Lot number:	ZZ15031, ZZ15033, ZZ15034, and ZZ15038
AF0150 Dosage (HDM):	20 mg/kg (52 fold PCD)

Specific Aim

To assess the hemodynamic and cellular responses to IV administration of AF0150 in anesthetized rabbits.

Methods

Animal Preparation: Twenty-seven New Zealand White rabbits (weighing 3.36±0.2 kg) were anesthetized with intravenous pentobarbital and divided into 3 groups. The first group (n=12), animals were intubated and mechanically ventilated on room air. The jugular vein was catheterized for drug injection and blood sampling, and the carotid artery for hemodynamic monitoring. The second group (n= 12), animals were instrumented with a catheter in the jugular vein only for drug injection and blood sampling. Third group (n=3, control group), animals were instrumented with two catheters (jugular vein and carotid artery), as in the first group, for hemodynamic monitoring and blood sampling.

AF0150 Preparation and Administration: AF0150 was reconstituted in sterile water to the concentration of 40 mg/ml. Animals in the first and second groups received a bolus injection of 20 mg/kg (0.5 ml/kg) AF0150 followed by a 1 ml flush of 0.9% saline through the jugular vein catheter. The control group (the third group) received a bolus injection of an equal volume of saline (0.5 ml/kg).

Observation Parameters: Heart rate and mean arterial pressure were measured before and 1, 5, 10, 20, 30, and 60 minutes after AF0150 injection. Blood samples were collected for determination of platelet and white blood cell (WBC) counts before and after 5, 10, 20, 30, and 60 minutes AF0150 injection using a Hematology Analyzer. At the end of the study, animals were sacrificed with an overdose of sodium pentobarbital.

Data Analysis: Data are expressed as Mean±SEM. Within group (from baseline) and between group differences were determined by two-way ANOVA test. The statistical significance level was defined at $p<0.05$.

RESULTS

Heart Rate and Mean Arterial Pressure (Table I)

IV injection 20 mg/kg AF0150 had no statistically significantly effects on heart rate (HR) and mean artery pressure (MAP) in the time-course (up to 60 minutes). There was also no statistic difference in HR and MAP between saline control and AF0150 treatment. However, AF0150 treatment groups appeared to decrease MAP at all time points including before AF0150 injection, although no statistically significant differences were noted.

Table I. Heart Rate (beats/min) and Mean Arterial Pressure (mmHg) in Anesthetized Rabbits Following Intravenous Injection of 20 mg/kg AF0150 or 0.5 mL/kg Saline

	Heart Rate		Mean Arterial Pressure	
	Control (n=3)	AF0150 (n=12)	Control (n=3)	AF0150 (n=12)
Baseline	283 ± 20	278 ± 9	101 ± 1	89 ± 6
1 min	280 ± 17	272 ± 9	102 ± 1	90 ± 6
5 min	280 ± 17	273 ± 9	101 ± 3	88 ± 5
10 min	272 ± 23	268 ± 11	101 ± 2	88 ± 5
20 min	267 ± 26	264 ± 8	100 ± 2	90 ± 4
30 min	260 ± 23	266 ± 9	99 ± 2	88 ± 4
60 min	253 ± 29	261 ± 10	101 ± 3	89 ± 4

Data are Mean ± SEM

Platelet and White Blood Cell Counts (Figure 1)

Platelet and WBC counts decreased approximately 35% and 31%, respectively, below baseline immediately following injection of AF0150. The changes returned to baseline levels at 30 for platelets and 20 minutes for WBC and were stable up to 60 minutes. There was no statistical significance for these changes/differences. The control animal did not show the corresponding change.

Discussion and Comments

AF0150 (20 mg/kg) IV injection had no significant effects on HR and MAP but transiently decreased both platelet and WBC counts as compared to before injection and saline-treated control rabbits. However, control study (n=3) was not conducted concurrently with AF0150 treatment study. The control was done in October 1994 while AF0150 was in July and August 1995. The higher MAP baseline in control animal compared with the AF0150 groups suggests that the experimental procedure variations and/or individual animal differences may be responsible. Therefore, the results from the control study were not comparable to the AF0150

treatment. The time-course results from AF0150 treatment studies are still considered adequate. The AF0150-induced transient decreases in platelets and WBC may be explained by complement activation, as the sponsor proposed. However, no corresponding hemodynamic changes were followed, which makes this possibility unlikely. It is possible that the AF0150 induced transient margination of those blood cells.

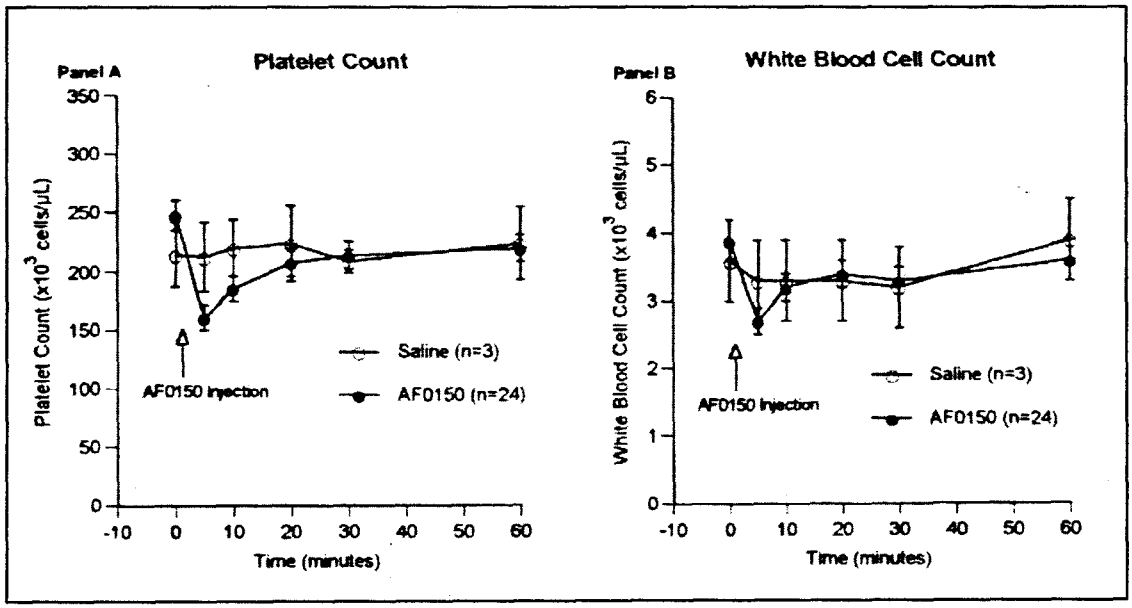

Figure 1. Effects of IV AF0150 injection on platelet count (panel A) and white blood cell count (panel B) in anesthetized rabbits. The animals received 20 mg/kg AF0150 or equal Volume of saline (0.5 ml/kg). Data are Mean ± SEM of 3 (control) or 24 (AF0150 treatment) rabbits.

Report Number: EB-97-13

Exposure of AF0150 to High Ultrasonic Power and the Effects on Hemolysis, Hematology and Hemodynamics

Report Location:	Vol. 009, p335-348
Report date:	September 17, 1998
Study Facility:	Alliance Pharmaceutical Corp.
In-life phase:	Not indicated
GLP Compliance:	No
AF0150 Lot number:	UA16064
AF0150 Dosage (HDM):	20 mg/kg (86 fold PCD), (1mg/kg/min infusion for 20 minutes)

Specific Aim

To evaluate the effects of AF0150 microbubbles in the presence of high ultrasonic power exposure on blood hemolysis, hematology and hemodynamics in normal dogs

Methods

Animal Preparation: Four male mongrel dogs (weighing 22.4, 21.8, 24.0, and 19.8 kg) were anesthetized with sodium pentobarbital and spontaneous respiration was maintained. Peripheral catheters were placed [*the site was not specified*] for collection of arterial blood samples, blood pressure measurements, and the administration of AF0150. An ultrasound probe was placed over the heart (closed chest) for ultrasound bursts.

AF0150 Preparation and Administration: AF0150 (200 mg fill) was reconstituted with 10 ml SWFI to a final concentration of 20 mg/ml. All animals received a 20-minute IV infusion of 1 mg/kg/min AF0150 (20 mg/kg).

Observation Parameters: Heart rate (HR) and blood pressure were monitored continuously. Arterial blood samples (2 ml) were collected for the hematology and hemolysis analysis before AF0150 injection in the presence or the absence of high ultrasound power (with MI 1.0 -1.1 for 15 min) bursts. All animals then received a 20-minute IV infusion of AF0150 while high ultrasound power was applied throughout the AF0150 infusion and continued for 10 minutes post-dosing. Arterial blood samples were collected at 15, 20, 30, 60, 120, 180, and 240 minutes after AF0150 administration. The hemolysis was determined on the basis of Hb release to plasma by a spectrophotometer, expressed as % of total hemolysis. Water-induced total hemolysis (*in vitro*) was defined as 100% hemolysis. Hematology measurements using a [redacted] Blood Count (CBC) blood analyzer include white blood cell (WBC), platelet (PLT), red blood cell counts (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), and mean plasma volume (MPV).

Data Analysis: Data are presented as Mean ± SEM without statistic analyses.

Results

One of the four dogs died after the administration of anesthesia, prior to infusion of AF0150, and data from this dog were not included in the analysis.

Hemodynamics (Figure 1): Heart rate was fairly constant throughout the study from 99±8 bpm to 108±4 bpm at 15 and 120 minutes following AF0150 administration, respectively. Blood pressure remained fairly constant throughout the 5-hour study from 131-145/87-120 mmHg.

Hematology (Figure 2): Platelet and WBC counts decreased within the first 15 minutes of AF0150 administration. The platelet counts returned to baseline (before AF0150 injection) by the end of study (4 hours), and the WBC count returned to baseline by 2 hours and was followed by a compensatory increase above the baseline. There was a great variation in WBC and platelet counts. There were no marked changes in RBC, HGB, HCT, MCV, MCH, MCHC, RDW and MPV.

Hemolysis: RBC hemolysis levels were <1% of total hemolysis at all time points (range of 0.17% to 0.64%) before, during and after AF0150 administration and ultrasound power exposure.

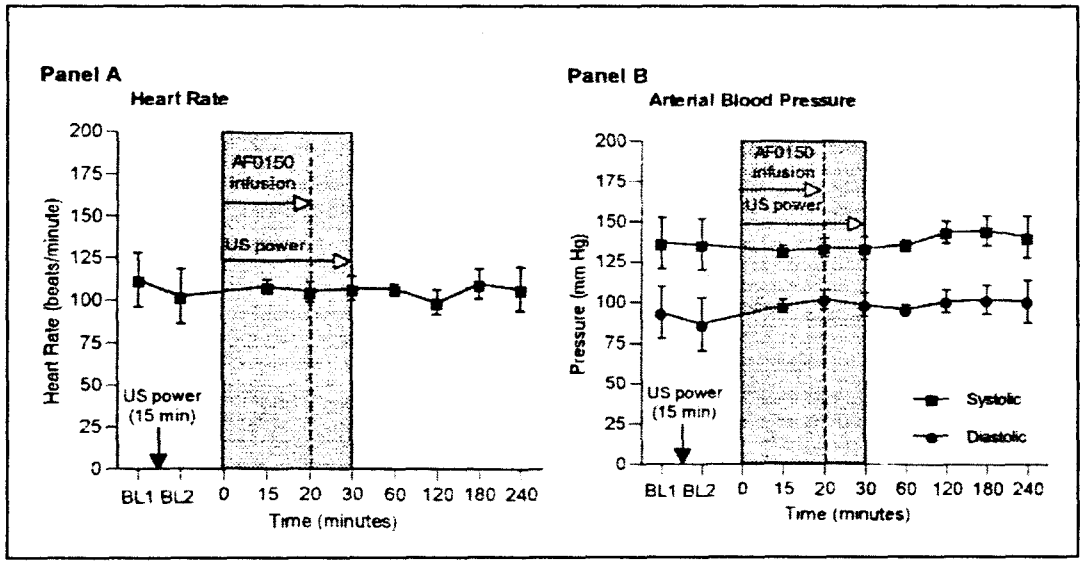

Figure 1. Heart rate (*Panel A*) and arterial blood pressure (*Panel B*) in anesthetized dogs before, during and after IV infusion of AF0150 (20 mg/kg) and high ultrasound (US) power burst. Data are Mean ± SEM of 3 dogs.

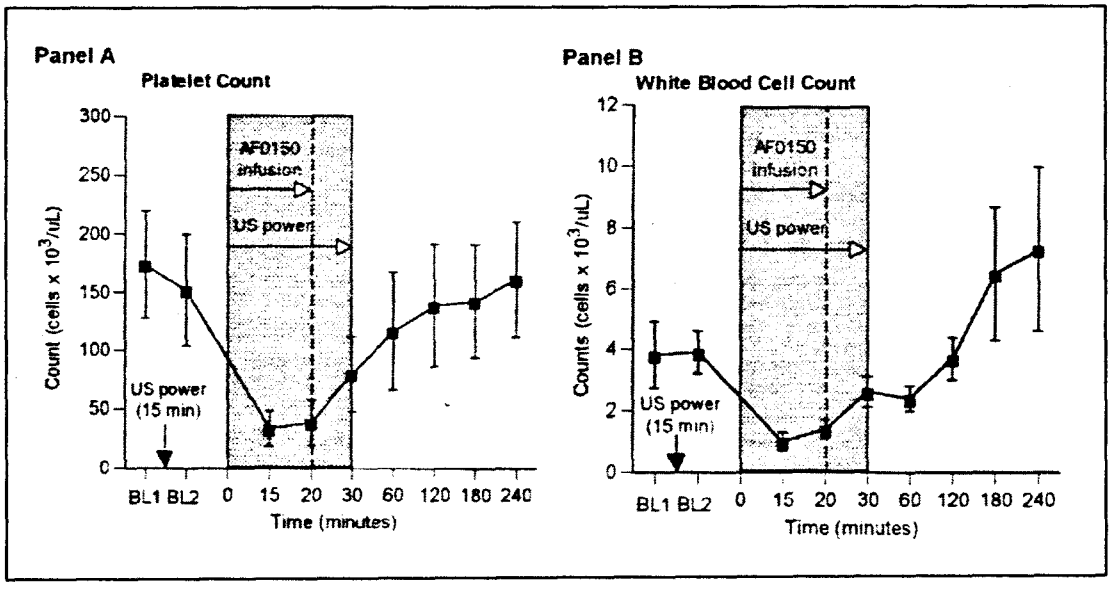

Figure 2. Platelet (*Panel A*) and WBC (*Panel B*) in anesthetized dogs before, during and after IV infusion of AF0150 (20 mg/kg) and high ultrasound (US) power burst. Data are Mean ± SEM of 3 dogs.

Discussion and Comments

1. In dogs, AF0150 at the dose of 20 mg/kg (1 mg/kg/min, 20-minute IV infusion) combined with high power ultrasound exposure had no significant effects on heart rate and arterial blood pressure, showed less than 1% hemolysis, but a transient decrease in platelet and WBC counts was noted. A similar effects on platelet was seen in a previous study in rabbits. The sponsor claimed that there was no such changes in the phase I clinical trials and thus proposed that species-difference may account for these findings.

2. In that hemolysis study, data from 2 dogs were presented without showing variation. The sponsor reported that several blood samples were not processed because extended incubation with anticoagulants prior to centrifugation, and separation of plasma resulted in hemolysis in the test tube. However, the data did not indicate any differences in this type of hemolysis as a results of AF0150 treatment and ultrasound exposure.

3. The blood cell counts in dog 97-1503A were lower than in the other two dogs. The platelet counts were half of that noted in the other dogs. An appropriate explanation needs to be provided.

Report Number: BS-95-27
Effects of AF0150 on Hemodynamics and Videointensity during Contrast Echocardiography in the Dog

Report Location:	Vol. 009, p349-362
Report date:	June 8 1999
Study Facility:	[redacted]
In-life phase:	November 1995-March 1996
GLP Compliance:	No
AF0150 Lot number:	ZZ15036
AF0150 Dosage (HDM):	0.4, 0.8, 1.2, 1.6 mg/kg (7 fold PCD)

Specific Aim

To determine the hemodynamic effects and contrast echocardiography characteristics of AF0150 (0.4 to 1.6 mg/kg)

Methods

Animal Preparation: Four hound dogs (16 to 18 kg) were anesthetized with IV pentobarbital and intubated with mechanical ventilation (tidal volume: 10 ml/kg, respiratory rate:15 breaths/minute, and a low level of positive end expiratory pressure). Via venous access, a Swan-Ganz catheter was used to measure cardiac output and pulmonary arterial pressure. The left femoral artery was catheterized for measurement of blood pressure and collection of samples (for

arterial O2 partial pressure). The left femoral vein was cannulated for AF0150 administration. Hear rate was monitored with ECG (chest leads).

AF0150 Preparation and Administration: AF0150 (200 mg fill) was reconstituted with 10 ml SWFI to a final concentration of 20 mg/ml. *The injection mode (bolus or infusion) and animal assignment (single dosing or sequential multiple dosing for each dog) were not specified in the study report, instead described a little in the Abstract.* Dogs received consecutive, incremental doses of AF0150 at 0.4, 0.8, 1.2, 1.6 mg/kg as a 5 second bolus injection followed by a 5 second saline flush (5 ml) through the left femoral vein catheter, with 20-25 minutes apart between injection.

Observation Parameters: heart rate, mean systemic arterial pressure, mean pulmonary arterial pressure were determined before (for baseline) and at 1, 3, 5, 10 minutes after each AF0150 injection. Cardiac output and arterial O2 partial pressure were measured before (for baseline) and at 3-5 minutes post AF0150 dosing. Transthoracic fundamental and second harmonic gray-scale cardiac ultrasound imaging was recorded using a 1.8 MHz transducer. The videointensity of left ventricular septum was analyzed in gray levels before (baseline) and at 10 seconds (fundamental only), 30 seconds, 1, 3, 5 minutes after AF0150 injection. It is not clear if echocardiography was performed at all dose.

Data Analysis: methods for data process and statistical analyses were not indicated in the report.

Results

Hemodynamics (Figure 1): compared to baseline values, AF0150 had no significant effects on heart rate, systemic arterial pressure, pulmonary arterial pressure, cardiac output at all doses and time points (up to 10 min post dosing).

Arterial O2 partial pressure: There were no significant changes in arterial oxygen partial pressure (PaO2) before and at 3-5 minute after AF0150 injection at all doses.

Echocardiography: AF0150 injection at all doses (0.4-1.6 mg/kg) increased videointensity of the left ventricular septum during fundamental continuous and second harmonic triggered imaging within first 1 minute post injection. Second harmonic triggered imaging produced improved videointensity compared to fundamental continuous imaging. However, the increases in the myocardial imaging did not show AF0150 dose-dependence during fundamental or second harmonic imaging.

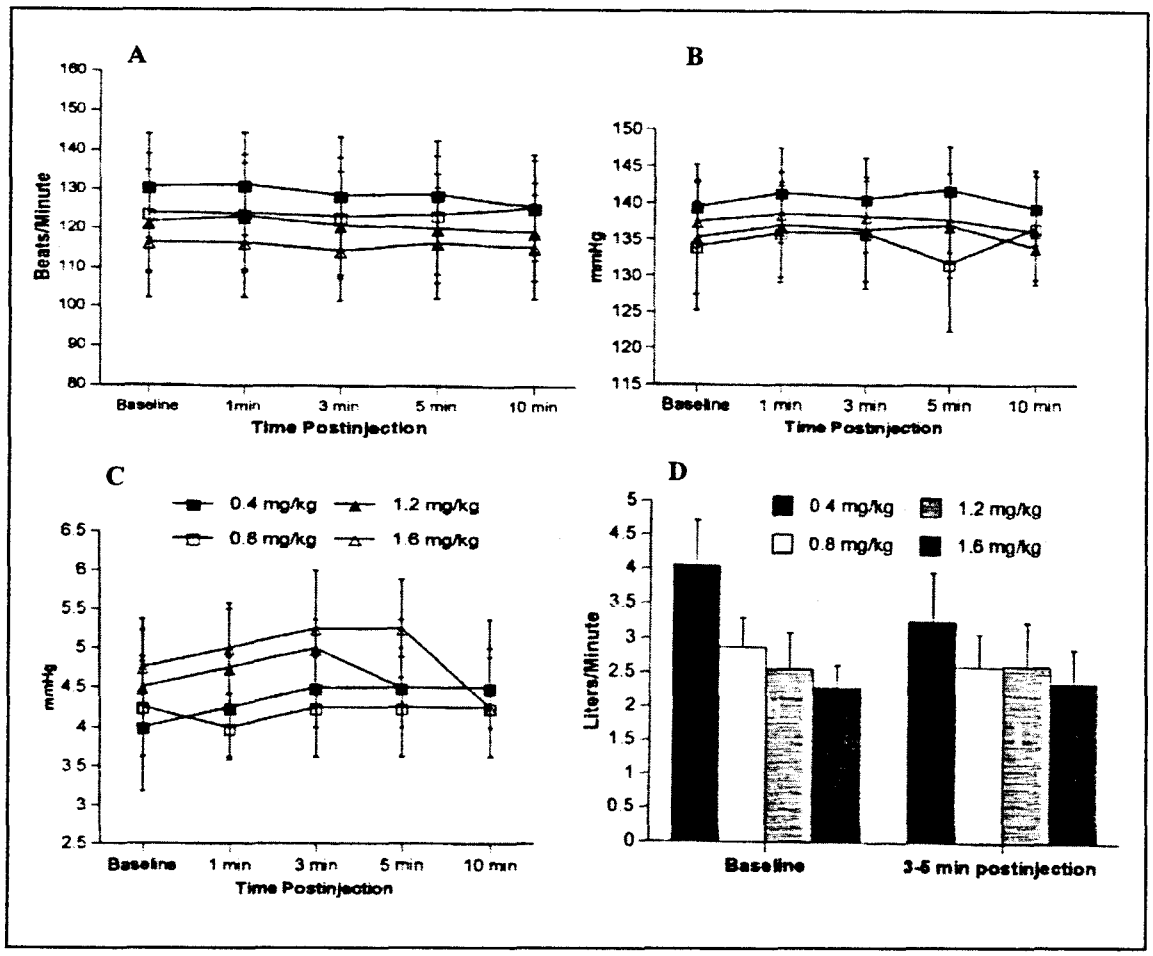

Figure 1. Effects of AF0150 IV injection on heart rate (***Panel A, beats/min***), systemic artery pressure (***Panel B, mmHg***), pulmonary artery pressure (***Panel C, mmHg***) and cardiac output (***Panel D, liter/min***) in anesthetized dogs. The baseline was measured at 20-25 minutes after each previous AF0150 injection. Data are Mean ± SEM (n=4).

Discussion and Comments

There were no significant differences in HR, artery BP, cardiac output and pulmonary artery pressure between before (baseline) and after AF0150 administration of 0.4-1.6 mg/kg (up to 10 minutes).

However, decreases in HR, artery BP and CO and increase in pulmonary artery BP at baseline were seen with incremental increase in AF0150 dose. Although anesthetics-induced effects may explain some changes, these baseline values were recorded 20-25 minutes after each previous AF0150 injection and thus carry-over of AF0150 was likely to result in the dose-dependent change in hemodynamics. Therefore, time-course following each single dose needs to be

observed. Also, AF0150-untreated dogs should be included in the study to control for anesthetic effects.

AF0150-induced myocardial imaging enhancement showed no dose-dependence in this study, which is inconsistent with the results presented in the General Pharmacology Studies. In previous studies, increases in videointensity were dose-dependent for LV myocardial imaging but dose-independence for LV cavity imaging. The appropriate explanation should be provided.

Report Number: EB-98-05
Effects of AF0150 on the Hemodynamic Response to Adenosine

Report Location:	Vol. 009, p363-374
Report date:	September 8 1998
Study Facility:	Alliance Pharmaceutical Corp
In-life phase:	Not specified
GLP Compliance:	No
AF0150 Lot number:	UA16038, ZY16042A
AF0150 Dosage (HDM):	2, 20 mg/kg (5-52 fold PCD)

Specific Aim

To evaluate the hemodynamic effects of AF0150 combined with the stress echocardiographic agent adenosine

Methods

Animal Preparation: Nineteen New Zealand White rabbits (3.40 ± 0.33 kg) were lightly sedated with an intramuscular injection of acepromazine (0.80 mg/kg). Ear arteries were cannulated for blood pressure measurements and blood sample collection. Ear veins were cannulated for infusion of test agents. Animals were randomly assigned to 2 groups and received adenosine at the doses of either 140 ug/kg/min (clinical dose, n=10) or 370 ug/kg/min (n=9).

AF0150 Preparation and Administration: AF0150 (100 mg fill) was reconstituted 10 ml of 0.45% sodium chloride to a final concentration of 10 mg/ml. Animals received 2 or 20 mg/kg AF0150 after adenosine administration (6-minute IV infusion to reach steady-state hemodynamic response).

Observation Parameters: blood gas, heart rate (HR), mean arterial pressure and routine hematology parameters were measured before and at 30 or 60 minutes after adenosine infusion, and after adenosine infusion plus AF0150 injection.

Results

Hemodynamics (Figure 1): Adenosine at 370 ug/kg/min, but not the human clinical dose of 140ug/kg/min, decreased mean arterial pressure (MAP) by 18% and increased heart rate (HR) increased by 30%. Administration of AF0150 at both 2 and 20mg/kg had no statistically significant effects on the hemodynamic responses.

Blood Gas Analysis and Hematology: blood samples were collected from only 140 ug/kg/min adenosine group combined with AF0150 injection up to 60 minutes after adenosine infusion. There were no remarkable changes in blood gas (PaO2, PaCO2, pHa and Base Excess) and blood cells (RBC, WBC, Hb, HCT and platelet) after adenosine infusion combined with or without AF0150 (2 and 20 mg/kg) administration.

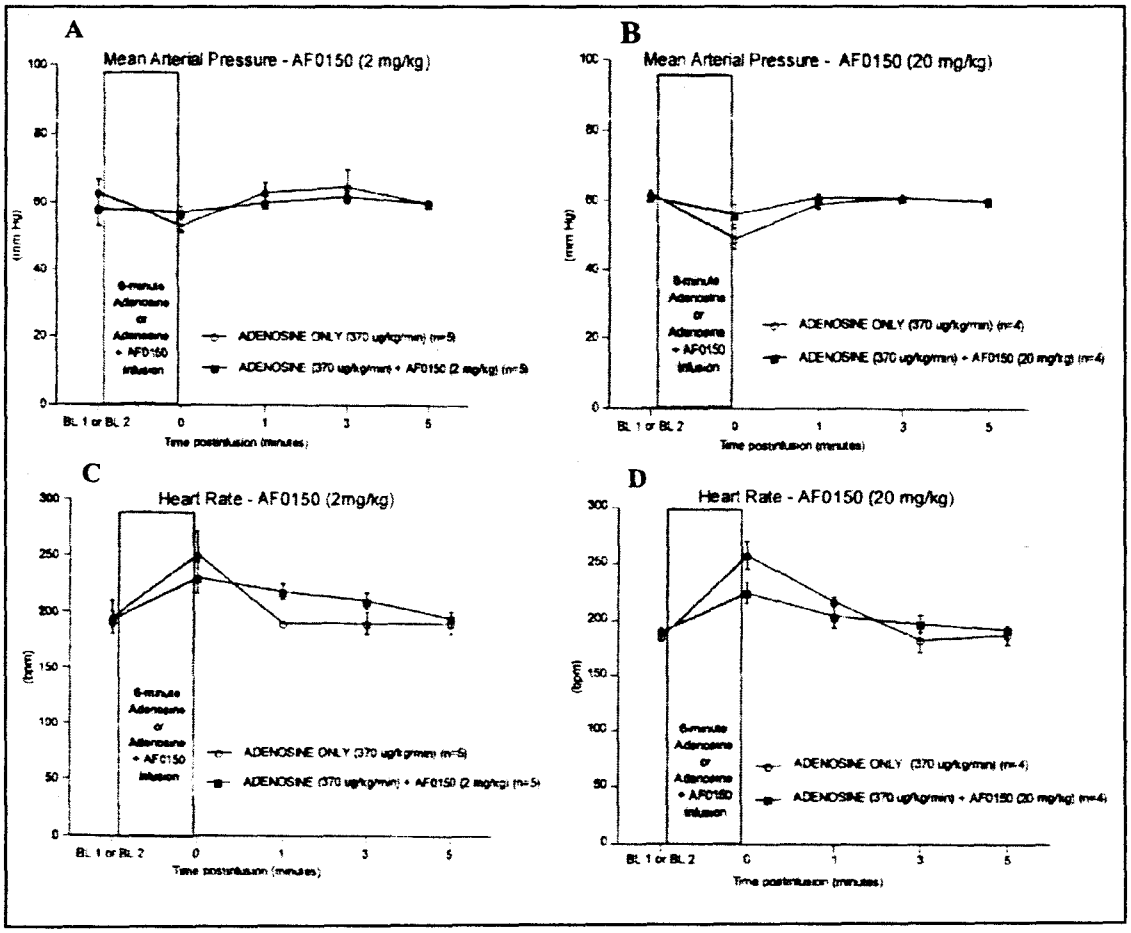

Figure 1. Effects of AF0150 on hemodynamics following adenosine-induced cardiovascular stress in rabbits. Animals received 370 ug/kg/min adenosine (6-minute IV infusion) and bolus IV injection of AF0150 (2 and 20 mg/kg). ***Panel A***, mean arterial pressure (mmHg) at 2 mg/kg AF0150; ***Panel B***, mean arterial pressure (mmHg) at 20 mg/kg; ***Panel C***, heart rate (beats/min) at 2 mg/kg; and ***Panel D***, heart rate (beats/min) at 20 mg/kg. Data are Mean±SEM of 4 or 5 animals.

Discussion and Comments

1. Adenosine at twice clinical dose (370 ug/kg/min, 6-minute IV infusion) only produced a slight cardiovascular response in rabbits, suggesting the adenosine-induced stress was not high enough to address the specific aim of this study. Blood gas and hematology analyses at the high dose of adenosine need to be provided to support the stress response. More observation parameters (such as cardiac output, ECG) are recommended to better address effects of AF0150 on the cardiovascular system in the presence of stress stimulation. On the other hand, different stress agents, particularly vasoconstriction agents, will be more suitable to test for potential adverse effects of the microbubbles (in terms of its physical properties) on the cardiovascular system.

2. The time-frame of adenosine and AF0150 administration was confusing. In the methods, AF0150 was administrated after "a peak steady-state hemodynamic response was achieved at approximately 6 minutes" (Vol.009, p367, Part IIB), however, in the Results, the AF0150 was given at the same time as the adenosine.

3. There were some editorial errors in Experimental Protocols. AF0150 was given by IV bolus injection but not IV infusion. However, the protocol kept saying "after AF0150 infusion" (Vol.009, p367). In the Part IA (Vol.009, p367), ".....collected at 0, 10, 20, 30, and 60 minutes after AF0150 infusion" should be "..... after adenosine infusion." or ".... after 0.45% saline (AF0150 vehicle) injection". In the Part IB ".... after AF0150 injection" instead of ".... after AF0150 infusion" should be used.

Report Number: EB-98-06
Effects of AF0150 on the Hemodynamic Response to Dipyridamole

Report Location:	Vol. 009, p375-389
Report date:	September 8 1998
Study Facility:	Alliance Pharmaceutical Corp
In-life phase:	No
GLP Compliance:	No
AF0150 Lot number:	UA16038, ZY16042A
AF0150 Dosage (HDM):	2, 20 mg/kg (5-52 fold PCD)

Specific Aim

To evaluate the effects of AF0150 on the hemodynamic response to a commonly used stress echocardiographic agent Dipyridamole

Methods

Animal Preparation: Four New Zealand White rabbits (3.41 ± 0.26 kg) were lightly sedated with an intramuscular injection of acepromazine (0.80 mg/kg) and ear arteries were cannulated for

pressure measurements and blood sample collection. Ear veins were cannulated for infusion of test agents.

AF0150 Preparation and Administration: AF0150 (100 mg fill) was reconstituted with 10 ml of 0.45% sodium chloride to a final concentration of 10 mg/ml. Animals received 2 and 20 mg/kg via IV bolus injection.

Observation Parameters: blood gas, hematology, and hemodynamic (mean arterial pressure and heart rate) measurements were measured before (baseline) and after dipyridamole infusion (142 ug/kg/min) or combined with AF0150 bolus injection. There was a 48 hours recovery period between the two AF0150 doses (2 and 20 mg/kg) combined with dipyridamole infusion. A steady-state hemodynamic response (where mean arterial pressure decreased and heart rate increased and both remained stable) was achieved at approximately 4 minutes.

Results

Hemodynamics (Figure 1): dipyridamole alone decreased mean arterial pressure by 18% and increased heart rate by 22% at 10 minutes after infusion. Co-administration of AF0150 had no effect on the mean arterial pressure, but potentiated the dipyridamole-induced tachycardia by approximately 18% at both AF0150 doses (2 and 20 mg/kg) at 10 minutes after AF0150 injection and returned to the baseline for Dipyridamole alone. The AF0150-induced HR increase was statistically significant as compared to dipyridamole infusion alone.

Blood Gases and Hematology: There were no significant changes in blood gases (pHa, PaO2, PaCO2 and base excess) and hematology parameters (WBC, RBC, Hb, HCT, Platelets) following dipyridamole only and AF0150 co-administration (up to 60 minutes post does).

Effects of propranolol pretreatment and aminophylline post-treatment on AF0150-induced hemodynamics in dipyridamole-treated rabbits (Appendix A, Vol.009, p386-389): a separate study was conducted in 4 rabbits (New Zealand, 3.41±0.26 kg). The animals were pretreated with 0.3 mg/kg propranolol followed by dipyridamole infusion or dipyridamole combined with AF0150 (2 or 20 mg/kg). Hemodynamics was observed for up to 60 minutes starting from propranolol administration. Aminophylline's effect was tested in 2 rabbits. 5 minutes after AF0150 co-administration with dipyridamole, the animals received 5 mg/kg aminophylline followed by collection of hemodynamic data starting from AF0150/dipyridamole administration. Propranolol pretreatment prevented and aminophylline reversed the AF0150-induced tachycardia without effects on mean blood pressure.

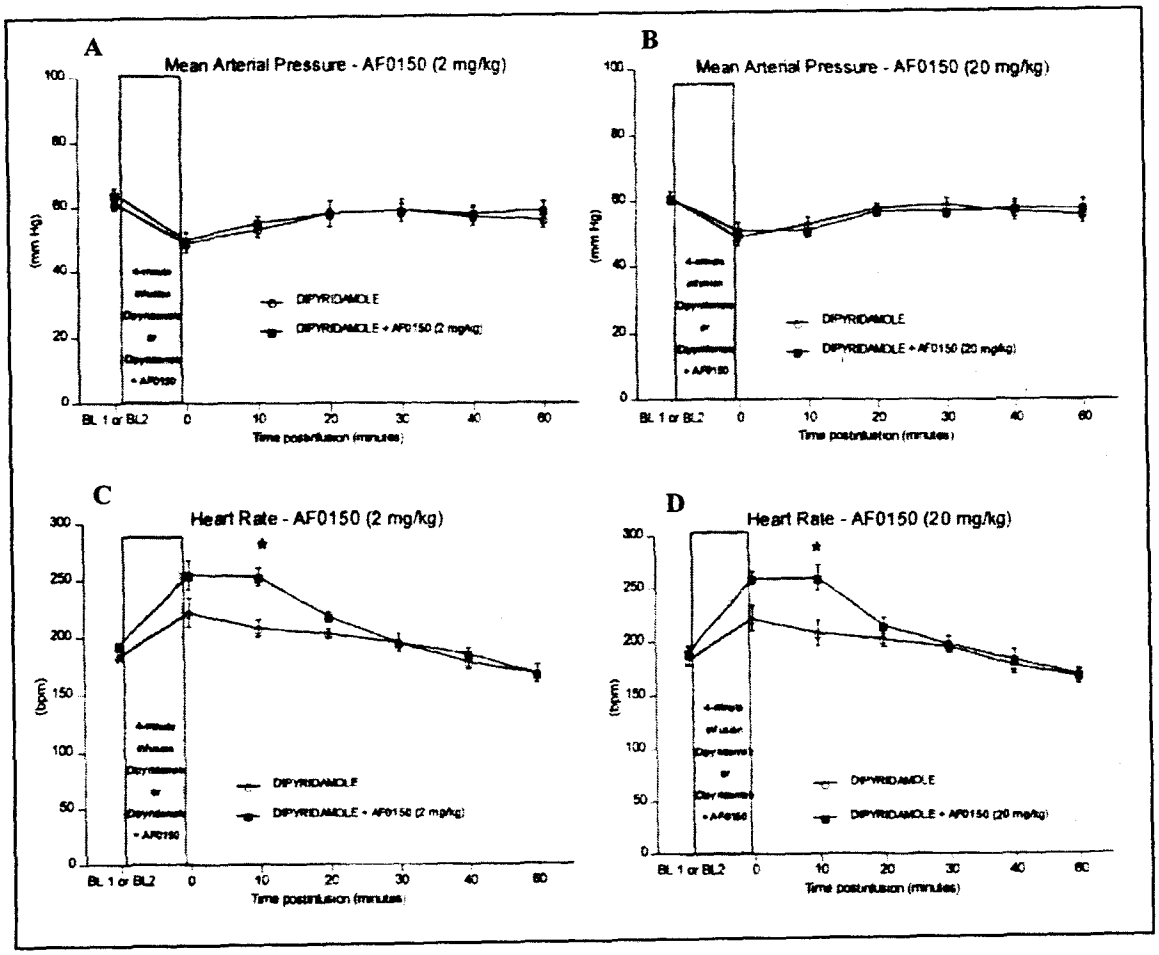

Figure 1. Effects of AF0150 on hemodynamics in dipyridamole-pretreated rabbits. Animals were pretreated with dipyridamole (142 ug/kg/min) by IV infusion for 4 minutes. AF0150 at 2 or 20 mg/kg was given by IV bolus injection. Mean arterial pressure (***Panels A and B***) and heart rate (***Panels C and D***) were monitored. Data were Mean ± SEM of 4 rabbits. * $P<0.05$ as compared with dipyridamole only.

Discussion and Comments

1. IV injection of AF0150 at the doses of 2 and 20 mg/kg transiently (lasting about 20 minutes) increased dipyridamole-induced tachycardia. The sponsor hypothesized that this effects was due to baroreflex and antagonizing adenosine receptors. Therefore, two supplemental studies were conducted with propranolol (beta-adrenergic receptor antagonist) pretreatment and aminophylline (adenosine receptor antagonist) post-treatment. Although propranolol prevented and aminophylline reversed AF0150-induced tachycardia, it is unlikely that the effects seen were due to baroreflex or adenosine receptor inhibition because AF0150 did not decrease the mean blood pressure and adenosine (in Study #EB-98-05) did not significantly increase HR. Likely, both agents functioned through physiological antagonisms to decrease the AF0150-induced increase in HR. The mechanisms of AF0150 effects on the heart need to be addressed by further studies.

2. The supplemental study was conducted separately from the main study. Observation made using AF0150 co-administration with dipyridamole in the absence of propranolol pretreatment was missing. It is hard to say that the propranolol truly prevented AF0150-induced effects without a concurrent control (AF0150/dipyridamole without propranolol).

Report Number: EB-98-07
Effects of AF0150 on the Hemodynamic Response to Arbutamine

Report Location:	Vol. 009, p390-398
Report date:	October 13 1998
Study Facility:	Alliance Pharmaceutical Corp
In-life phase:	December 1997 – January 1998
GLP Compliance:	No
AF0150 Lot number:	UA16063
AF0150 Dosage (HDM):	2, 20 mg/kg (5-52 fold PCD)

Specific Aim

To assess the effects of AF0150 microbubbles on the hemodynamics in rabbits treated with pharmacological stress agent arbutamine (increasing heart rate, cardiac contractility and systolic blood pressure).

Methods

Animal Preparation: Four New Zealand White rabbits (3.51±0.16kg) were lightly sedated with an intramuscular injection of acepromazine (0.80 mg/kg). Ear arteries were cannulated for measuring blood pressure and heart rate, and ear veins were cannulated for infusion of test agents.

AFO150 Preparation and Administration: AF0150 (100 mg fill) was reconstituted with 10 ml of 0.45% sodium chloride at a final concentration of 10 mg/ml. IV bolus injection of 2 or 20 mg/kg AF0150 was given at approximately 1 to 2 minutes after steady-state hemodynamic response (100 ng/kg/min IV infusion of arbutamine for 5-6 minutes). A 48-hours recovery period was allowed between the two AF0150 injection.

Observation Parameters: the mean arterial blood pressure and the heart rate were recorded through a cannulated ear artery before and up to 15 minutes after arbutamine infusion or after combined administration with AF0150. The timeline of AF0150 administration and data collection is as follows:

BL1	**Arbutamine** (100 ng/kg/min) ~5-6 minutes	Data Collection *15 minutes* / Recovery 30 minutes	BL2	**Arbutamine** (100 ng/kg/min) + **AF0150** (2 mg/kg) ~7-8 minutes	Data Collection *15 minutes* / Recovery ~48 hrs	BL3	**Arbutamine** (100 ng/kg/min) + **AF0150** (20 mg/kg) ~7-8 minutes	Data Collection *15 minutes*

Results

As seen in *Figure 1*, arbutamine infusion resulted in increase in heart rate by 32% and persisted for about 10 minutes [*Not specified if it was at the end of infusion*] but did not change mean blood pressure. Co-administration of AF0150 at both 2 and 20 mg/kg had no effects on heart rate and mean blood pressure.

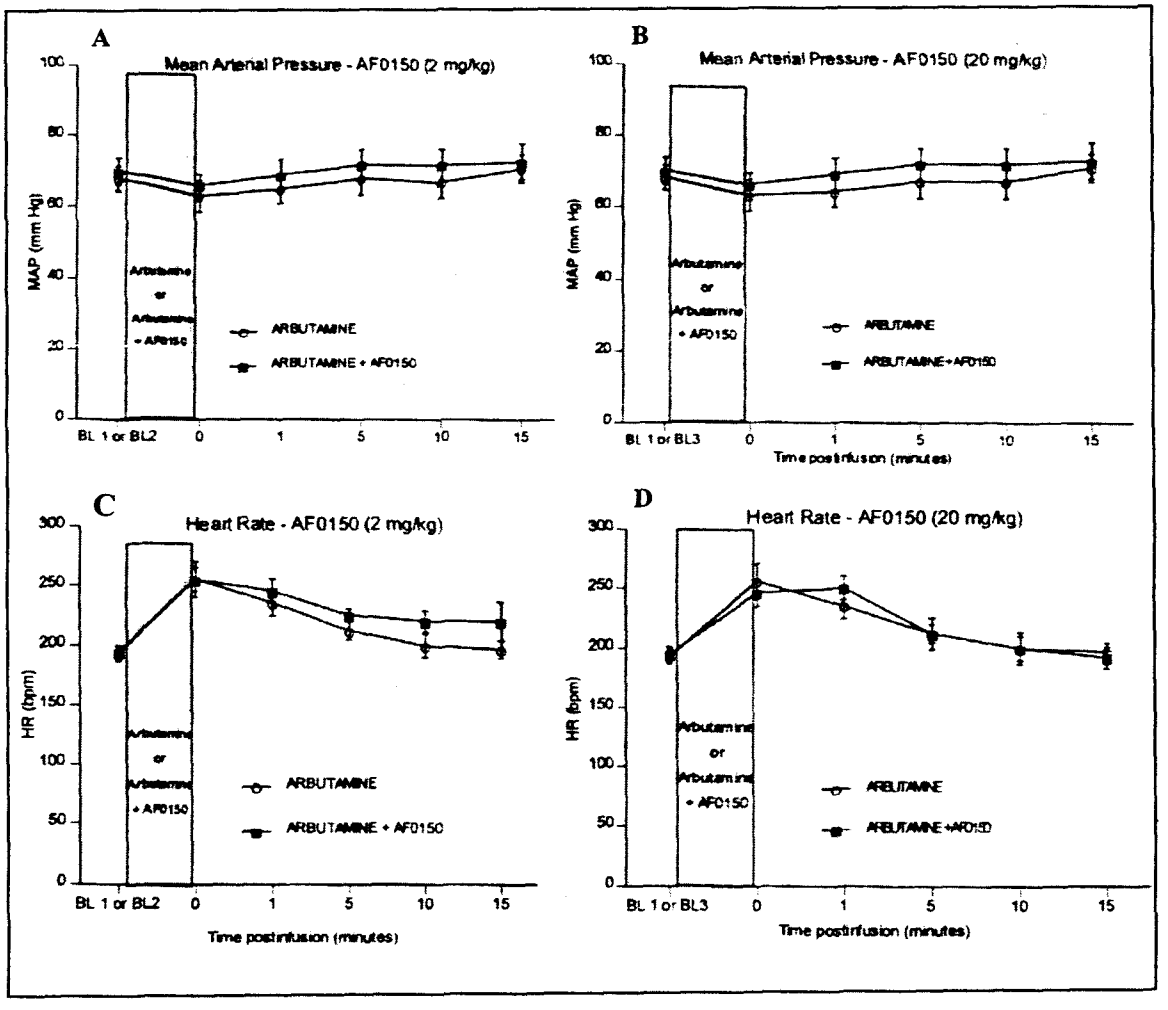

Figure 1. effects of Af0150 microbubble on hemodynamics in arbutamine-pretreated rabbits. Animals received IV infusion of 100 ng/kg/min arbutamine followed by IV bolus injection of AF0150 (2 or 20 mg/kg). Mean arterial blood pressure (***Panels A and B***) and hear rate (***Panels C and D***) were measured through ear artery catheter. Data are Mean ± SEM of 4 animals.

Discussion and Comments

The results are not consistent with findings in the dipyridamole-pretreated rabbit study (EB-98-06) in which AF0150 increased dipyridamole-induced tachycardia. Again, more sensitive and specific cardiovascular measurements (ECG, cardiac imaging, etc.) are necessary to accurately

monitor stress responses and address AF0150's effects. It is also necessary to test if ultrasound application alter the pharmacological response of AF0150 combined with the stress agents.

Report Number: EB-98-08
Effects of AF0150 on the Hemodynamic Response to Dobutamine

Report Location:	Vol. 009, p399-408
Report date:	October 13 1998
Study Facility:	Alliance Pharmaceutical Corp
In-life phase:	August 1998
GLP Compliance:	No
AF0150 Lot number:	UA16038
AF0150 Dosage (HDM):	2, 20 mg/kg (5-52 fold PCD)

Specific Aim

To assess the effects of AF0150 microbubbles on the hemodynamics in rabbits treated with pharmacological stress agent dobutamine (increasing heart rate, cardiac contractility and systolic blood pressure).

Methods

Animal Preparation: Four New Zealand White rabbits (3.15±0.1 kg) were lightly sedated with an intramuscular injection of acepromazine (0.80 mg/kg). Ear arteries were cannulated for hemodynamic measurement and blood sample collection, and ear veins were cannulated for administration of test agents.

AF0150 Preparation and Administration: AF0150 (100 mg fill) was reconstituted with 10ml of 0.45% sodium chloride to a final concentration of 10 mg/ml. IV bolus injection of 2 or 20 mg/kg AF0150 was given at approximately 1 to 2 minutes after steady-state hemodynamic response (5, 10, 20, 30 and 40 ug/kg/min IV infusion of dobutamine for 15 minutes, 3 minute between each incremental doses). A 48-hours recovery period was allowed between each of the two AF0150 injections.

Observation Parameters: Hemodynamic (mean arterial pressure and heart rate), blood gas (PaO2, PaCO2, pH) and hematology (platelets, WBC) before and up to 60 minutes after dobutamine infusion or after combined administration with AF0150. The timeline of AF0150 administration and data collection is as follows:

BL1	**Dobutamine** (5-40 µg/kg/min) *~15 minutes*	Data Collection *60 minutes* / Recovery 60 minutes	BL2	**Dobutamine** (5-40 µg/kg/min) + **AF0150** (2 mg/kg) *~17 minutes*	Data Collection *60 minutes* / Recovery ~48 hrs	BL3	**Dobutamine** (5-40 µg/kg/min) + **AF0150** (20 mg/kg) *~17 minutes*	Data Collection *60 minutes*

Results

Hemodynamics (Figure 1): Dobutamine alone increased heart rate (HR) by 32% without effect on mean arterial pressure (MAP). Combined administration of AF0150 had no effect on HR and MAP at 2 mg/kg but slightly increased HR and MAP at 20 mg/kg AF0150.

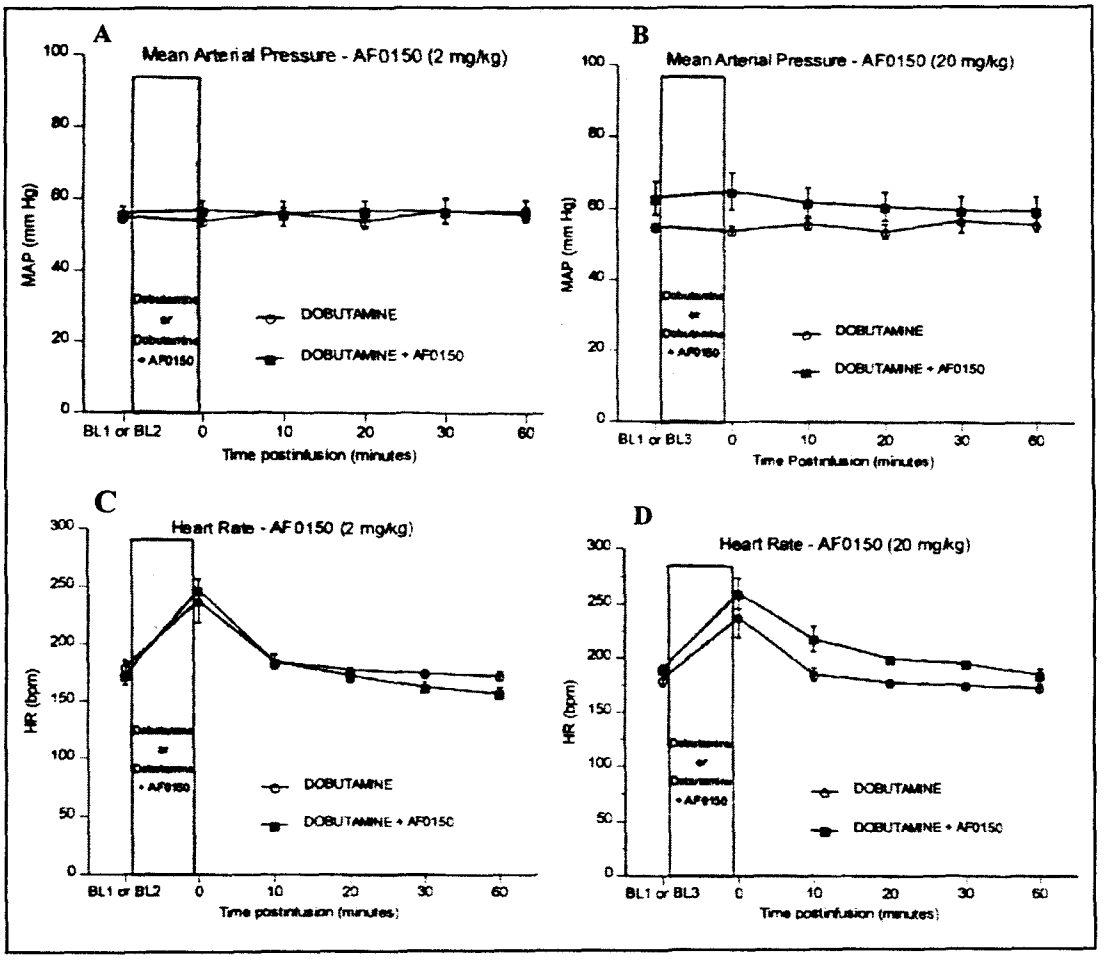

Figure 1. Effects of AF0150 microbubbles on hemodynamics in dobutamine-pretreated rabbits. Animals received IV infusion of 5-40 ug/kg/min dobutamine followed by IV bolus injection of AF0150 (2 or 20 mg/kg). Mean arterial blood pressure (***Panels A and B***) and heart rate (***Panels C and D***) were measured through an ear artery catheter. Data are Mean ± SEM of 4 animals.

Blood Gas and Hematology: no remarkable changes in blood gas and blood cells after administration of dobutamine alone and combination with AF0150.

Discussion and Comments

AF0150 at high dose (20 mg/kg) slight increased dobutamine-induced tachycardia and high mean arterial pressure, although there was no statistical significance. The increased HR is consistent

with the finding in dipyridamole pretreatment study (EB-98-06). It is likely that AF0150 microbubbles stimulate, at least transiently, cardiac activity when the heart is under stress. However, as commented in previous study reviews, the CV parameters were not sensitive and specific enough to address the potential effects of AF01050m microbubble on stressed heart. And the effects of AF0150 with ultrasound application and the stress agents need to be addressed.

Report Number: EB-98-13

Effects of AF0150 on Pulmonary Artery Pressure in a Rabbit Model of Pulmonary Hypertension

Report Location:	Vol. 009, p409-421
Report date:	October 13 1998
Study Facility:	Alliance Pharmaceutical Corp
In-life phase:	March-May 1998
GLP Compliance:	No
AF0150 Lot number:	ZZ17054
AF0150 Dosage (HDM):	1, 4, 10 mg/kg (2.6-26 fold PCD)

Specific Aim

To assess the effects of AF0150 microbubbles on pulmonary artery pressure (PAP) in rabbits with normal pulmonary pressure and with thromboxane A2-induced pulmonary hypertension.

Methods

Animal Preparation: Eleven New Zealand White rabbits (3.49±0.13 kg) were anesthetized by intramuscular injection with a mixture of ketamine, acepromazine, and xylazine. Central ear arteries were cannulated for arterial blood pressure and blood sample collection. Ear veins and jugular veins were cannulated for infusion of test substances. Animals were tracheotomized and mechanically ventilated to maintain arterial oxygen tension (PaO2) levels above 100 mmHg and arterial carbon dioxide tension (PaCO2) levels at 35-45 mmHg. Animals then underwent a right lateral thoracotomy and a catheter was placed into the pulmonary artery, via the right ventricle, for pulmonary artery pressure measurements. The pulmonary hypertension was reproduced by continuous intravenous infusion of thromboxane A analog (U46619) throughout the study. Mild increases in PAP (with systolic PAP 45% above baseline) and moderate increases in PAP (with systolic PAP 60% above baseline) were achieved by controlling infusion rate of U46619.

AF0150 Preparation and Administration: AF0150 (100 mg fill) was reconstituted in 10 ml of 0.45% NaCl to a final concentration of 10 mg/ml. Animals at each group, normal (n=3), mild hypertension (n=4) and moderate hypertension (n=4), received sequential bolus IV injection of 1, 4, 10 mg/kg AF0150 with a 10-min interval after baseline recording.

Observation Parameters: Mean arterial pressure (MAP), heart rate (HR), pulmonary artery pressure (PAP) and arterial blood gas (PaO2, PaCO2, pHa) were monitored before and after each AF0150 injection. The PAP was measured at 1, 5, 10 minutes; MAP/HR at 10 minutes post dosing (1-10 mg/kg) and blood gas at 10 minutes post dosing (10 mg/kg).

Results

Hemodynamics: AF0150 administration had no remarkable effects on the mean systolic PAP (*Figure 1*), MAP (*Figure 2*) and HR in normal rabbits (with normal pulmonary artery pressure, approximately 10 mmHg) and pulmonary hypertension (both mild and moderate). However, the MAP baseline increased in both mild and moderate hypertensive rabbits.

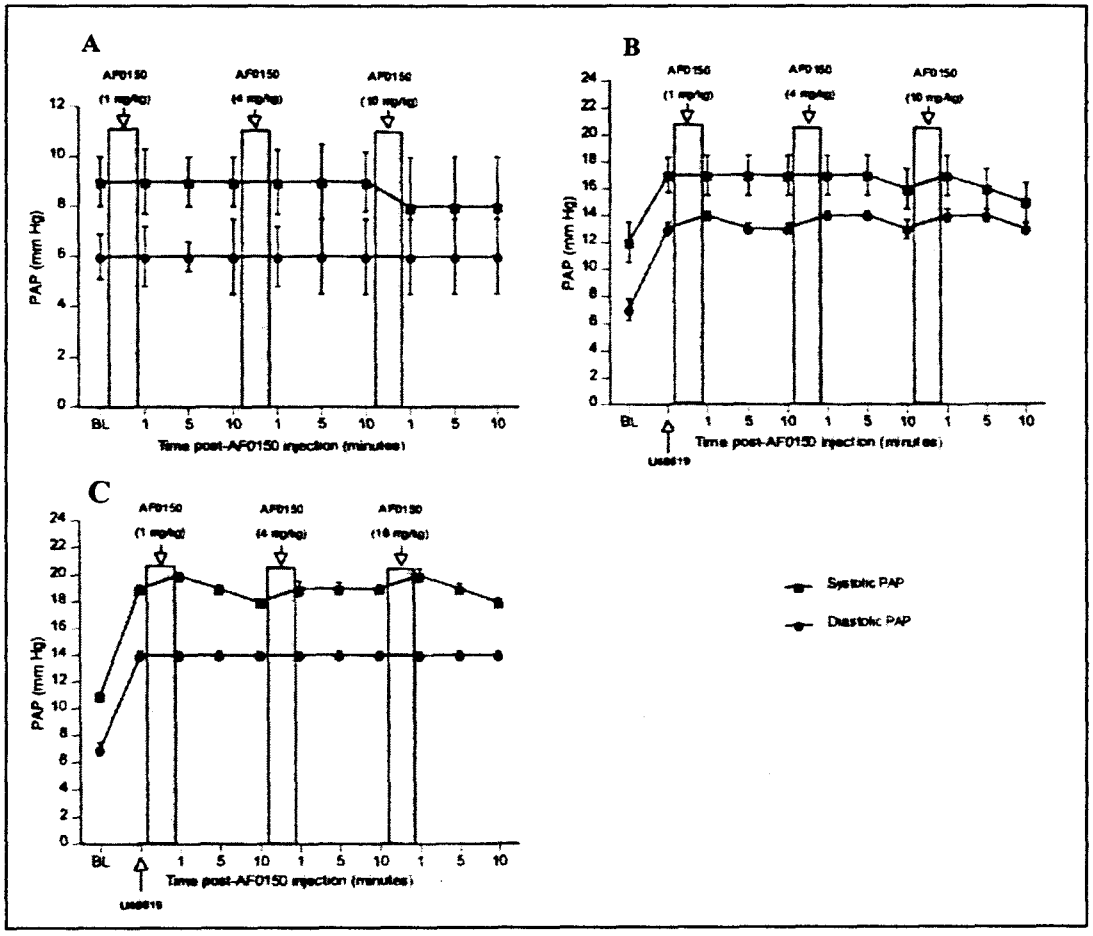

Figure 1. Effects of AF0150 IV injection on pulmonary artery pressure in normal and U46639-induced pulmonary hypertensive rabbits. ***Panel A,*** normotensive group (n=3); ***Panel B,*** mild hypertensive group (n=4); ***Panel C,*** moderate hypertensive group. Data are Mean±SEM.

Blood Gases: AF0105 Administration had no remarkable effects on PaO2, PaCO2 and pH in the pulmonary normotensive and hypertensive rabbits. However, base excess was 73% less in the

mild hypertensive rabbits and 47% less in the moderate hypertensive rabbits than in the normotensive group at 10 minutes after injection of 10 mg/kg AF0150.

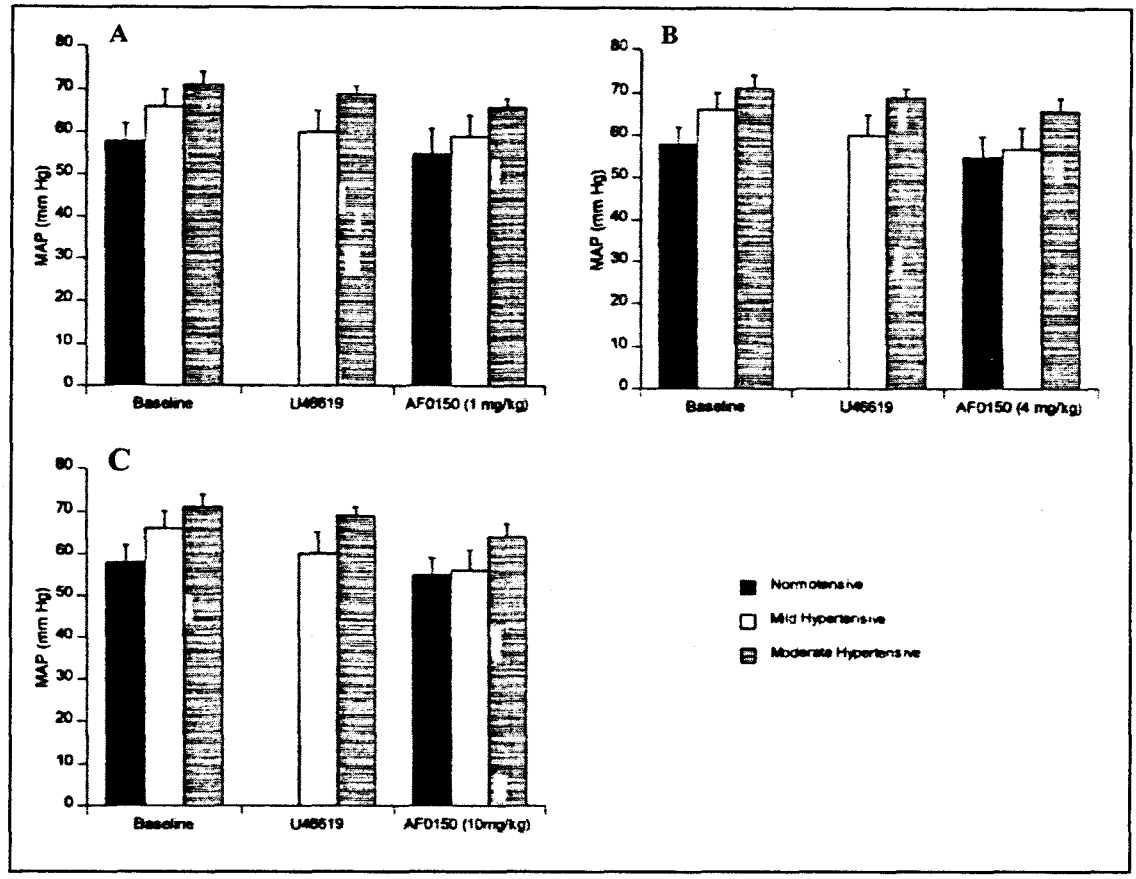

Figure 2. Effects of AF0150 IV injection on mean artery blood pressure (MAP) in normal and U46619-induced pulmonary hypertensive rabbits. ***Panel A,*** 1 mg/kg AF0150; ***Panel B,*** 4 mg/kg AF0150; ***Panel C,*** 10 mg/kg AF0150. Data are Mean±SEM of 3 (for normal group) or 4 (for hypertensive groups).

Discussion and Comments

1. IV administration of AF0150 at the doses of 1-10 mg/kg had no significant effects on pulmonary artery pressure in normal rabbits and in U46619-induced pulmonary hypertensive rabbits during the 10 minutes observation. However, no positive controls were used to validate the assay system.

2. In a pre-NDA meeting held on July 29th 1999, the sponsor was informed that microspheres and/or direct injection of AF0150 into the right ventricle or the pulmonary artery needed to be included as positive controls. This would increase the sensitivity for evaluation of the effects of the microbubbles on the pulmonary circulation.

3. Significant decrease in blood base excess after 10 mg/kg AF0150 treatment was observed in the hypertensive rabbits. An appropriate explanation was not provided, even though this issue was raised at the pre-NDA meeting (July 29th 1999).

4. The reference article cited in this study does not have direct relevance to this study. This may be an editorial error because other articles in the same issue of *Chest* are more relevant to this study, such as "Lipid mediator dysregulation in primary pulmonary hypertension" (Chest 114 (3): p205s-207s, 1998).

5. The baseline MAP increased in the U46619-induced pulmonary hypertensive rabbits, which may interfere with AF0150's effects. An appropriate explanation was not provided in the report.

Report Number: BS-97-09
Effect of AF0150 on the Nuclear Imaging of 99mTC-Sestamibi

Report Location:	Vol. 010, p016-028
Report date:	October 12, 1998
Study Facility:	Alliance Pharmaceutical Corp
In-life phase:	May 1997
GLP Compliance:	Not specified
AF0150 Lot number:	ZZ16006
AF0150 Dosage (HDM):	0.5, 2.0 mg/kg (1.3-5.2 fold PCD)

Specific Aim

To determine if AF0150 administration would interfere with the diagnostic efficacy of nuclear imaging of the radioactive tracer 99mTc-Sestimibi in normal and ischemic myocardial tissue.

Methods

Animal Preparation: Thirty-two New Zealand White rabbits (BW 3.8±0.4 kg) of either sex were anesthetized with an initial dose of 30 mg/kg pentobarbital and supplemented as needed. Animals were intubated with mechanical ventilation to maintain blood gases within physiological range. Animals were instrumented for arterial blood sampling, ECG (Lead II) and blood pressure measurements. All animals were subject to thoracotomy and heart exposure, and were randomly divided into six groups:

Normal Groups
Control (n = 4) without AF0150 injection
0.5 mg/kg AF0150 (n = 6)
2.0 mg/kg AF0150 (n = 6)

Occlusion Groups
Control (n = 4) without AF0150 injection
0.5 mg/kg AF0150 (n = 6)
2.0 mg/kg AF0150 (n = 6)

In the "occlusion" groups, animals received procainamide (10 mg/kg, intramuscular) to prevent cardiac arrhythmia, and the left circumflex artery (LCX) was ligated at the juncture with the left-main coronary artery to decrease perfusion of the left ventricular free wall. All animals received an intravenous infusion of the vasodilator dipyridamole (0.56 mg/kg) via syringe pump followed by administration of 99mTc-Sestimibi, and AF0150 (0.5 or 2.0 mg/kg) or saline (control groups). Serial nuclear imaging of the heart was performed for 30 minutes. Monastral blue dye (1.0 ml) was finally injected into the left ventricular cavity to delineate the area of infarct. At the conclusion of the study, all animals were euthanized with pentobarbital and saturated KC1 and the heart was excised and sliced into transverse sections. Myocardial samples from the left ventricular free wall (infarct) and septal regions (normal perfusion) of the left ventricle were biopsied, weighed, and stored for analysis of total MIBI content. Midventricular tissue samples were collected for analysis of area at risk and area of infarct determination.

AF0150 Preparation and Administration: AF0150 (200 mg fill) was reconstituted with 10 ml SWFI to a final concentration of 20 mg/ml. Animals received IV bolus injection of 0.5 or 2.0 mg/kg AF0150.

Observation Parameters: Hymodynamics (heart rate and mean arterial pressure) were recorded before and after dipyridamole and 30-min imaging, reported as mean of 5 consecutive cycles. Area at Risk (myocardial area delineated by the blue dye distribution), Area of Infact (myocardial area indicated by MIBI that was nonperfused and nonviable), MIBI Tissue Content (gamma counts per myocardial tissue weight) and MIBI Washout (% washout of MIBI from normal and occluded regions) were measured with biopsy at the end of the experiment.

Results

Hemodynamics (Table 1): IV injection of AF0150 at 0.5 and 2.0 mg/kg had no remarkable effects on heart rate and mean blood pressure in rabbits with normal myocardial perfusion and in rabbits with experimental ischemia, either in the presence or the absence of vasodilator dipyridamole before and after MIBI imaging.

Myocardial Perfusion/Ischemia: AF0150 caused no changes on the area at risk and area of infarct after LCX ligation. MIBI tissue content decreased in ischemic groups and AF0150 did not affect the MIBI tissue content in both normal and ischemic rabbits. MIBI tissue washout (the less, the higher myocardial perfusion) showed less washout in normal (28%) than in ischemic rabbits (45%) and AF0150 administration had no effects on the washout in both normal and ischemic groups.

Table 1. Effects of AF0150 on heart rate and mean arterial pressure before and after dipyridamole infusion and MIBI imaging in rabbits with normal and experimental ischemic myocardium.

Group	Heart Rate (bpm)			Mean Arterial Pressure (mm Hg)		
	Baseline	*Dipyridamole*	*Imaging*	*Baseline*	*Dipyridamole*	*Imaging*
Normal Groups						
Control (n=4)	252 ± 9	258 ± 12	252 ± 8	86 ± 2	53 ± 7	72 ± 9
0.5 mg/kg AF0150 (n=6)	264 ± 5	274 ± 3	250 ± 3	90 ± 1	49 ± 9	77 ± 5
2.0 mg/kg AF0150 (n=6)	274 ± 6	286 ± 7	270 ± 8	90 ± 2	63 ± 5	80 ± 4
Occlusion Groups						
Control (n=4)	252 ± 13	253 ± 10	237 ± 13	86 ± 2	46 ± 2	66 ± 5
0.5 mg/kg AF0150 (n=6)	247 ± 5	251 ± 10	234 ± 11	84 ± 2	52 ± 4	62 ± 5
2.0 mg/kg AF0150 (n=6)	258 ± 4	258 ± 7	239 ± 4	84 ± 2	52 ± 6	58 ± 7

Discussion and Comments

This study was originally designed to test if AF0150 administration interferes with 99mTc-Sestimibi (MIBI) myocardial imaging because in the clinical setting both procedures may used in the same patients. It seems that AF0150 had no effects on MIBI imaging. However, this study should be designed better to evaluate AF0150 effects on ischemic myocardium using the MIBI imaging and/or radioactivity counting as a tool rather than to test efficacy. It would be more valuable for cardiac safety assessment if the study was designed with a longer (instead of 30 minutes) observation time post AF0150 dosing, more dose groups of AF0150 and more detailed myocardial perfusion examination.

There are editorial errors in the lines 9 and 11 under Results (Vol.010, p022), the unit for MAP should be mmHg (89, 62 and 76 mmHg).

Report Number: IMUS-016-TOX

An Evaluation of Hemodynamic Changes in Conscious Cynomolgus Monkeys Following Intravenous Administration of AF0150

Report Location: Vol.022, p182-286
Report date: January 26, 1996
Study Facility: [redacted]
In-life phase: October 2-23, 1995
GLP Compliance: Yes (with QA Statement)
AF0150 Lot number: ZZ15036
AF0150 Dosage (HDM): 10, 20, 40 mg/kg (26-104 fold PCD)

Specific Aim

To evaluate effects of AF0150 IV bolus on hemodynamics in conscious cynomolgus monkeys

Methods

Animal Preparation: eight male cynomolgus monkeys were obtained from [redacted] with body weights of 3.9-5.3 kg at initiation of treatment ([*age was not provided*]). The monkeys were held in quarantine prior to initiation of treatment for 2 weeks, including TB test, physical examination, hematology, blood chemistry analyses, rectal swab, feces test (for parasites). Standard procedures were followed for housing, handling, feeding and care of primates. The animals were randomly assigned to 2 groups (4/group), as seen in Table 1.

Surgical Operation: Femoral vein and arteries of each monkey were catheterized under anesthesia for pulmonary arterial pressure (PAP), cardiac output (CO), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), left ventricular pressure (LVP) and derived contractility (dp/dt). Briefly, the catheterization followed the routine surgical procedures for primates. Through femoral veins a catheter was placed to the caudal vena cava for collection of blood samples, and a thermistor-tipped catheter was placed in the pulmonary artery for measurement of CO and PAP. Through femoral arteries, a catheter was placed to the aorta for measurement of SAP, DAP and MAP, and to the left ventricle for the measurement of LVP and dp/dt. All catheters were brought subcutaneously to the exteriorization point in the midscapular region. All surgical sites were closed using appropriate suture materials. Continuous IV infusion of heparin/saline (at 1 ml/hour) maintained catheter patency till initiation of treatment. All catheters were infused ([*filled?*]) with heparinized saline (2 U/ml) following surgical procedure and throughout the hemodynamic measurements. The animals were allowed to recover from the surgery for one hour or longer (until stabilization of hemodynamic parameters was achieved) prior to initial treatment.

Table 1. Hemodynamic study procedure in monkeys

Group Number	Number of Animals	Treatment Administration					Observation Period
		Substance	Dose Level (mg/kg)	Dose Volume (mL/kg)	Route	Dosing Regimen	
1	4	Saline	0	0.5	IV (bolus)	Once for each dose level	At least 1 hr after each treatment
			0	1.0			
			0	2.0			
2	4	AF0150	10	0.5			
			20	1.0			
			40	2.0			

IV = intravenous injection.

AF0150 Preparation: AF0150 (200 mg fill vial) was reconstituted with 10 ml of sterile water to a concentration of 20 mg/ml and used within 30 minutes of preparation. The animals received 10, 20, and 40 mg/kg AF0150, once each dose, by a bolus IV injection into the saphenous vein via a percutaneous catheter when animals were conscious (after recovered from anesthesia) and retained in a sling.

Observation Parameters: Clinical signs were observed during treatment and monitoring period. Body weight was recorded prior to surgical procedure. Hemodynamic monitoring and sample collection was shown in Table 2.

Table 2. Hemodynamic Monitoring and Sample Collection Schedule

Time	Hemodynamic parameters									Clinical Pathology
	ECG	HR	PAP	SAP	DAP	MAP	LVP	dp/dt	CO	Hematology
Prior to initiation of first treatment[1]	X	X	X	X	X	X	X	X	X	X
2 mins	X	X	X	X	X	X	X	X	X	–
5 mins	X	X	X	X	X	X	X	X	X	X
10 mins	X	X	X	X	X	X	X	X	X	X
15 mins	X	X	X	X	X	X	X	X	X	–
30 and 60 mins	X	X	X	X	X	X	X	X	X	X
Prior to initiation of second treatment[1]	X	X	X	X	X	X	X	X	X	X
2 mins	X	X	X	X	X	X	X	X	X	–
5 mins	X	X	X	X	X	X	X	X	X	X
10 mins	X	X	X	X	X	X	X	X	X	X
15 mins	X	X	X	X	X	X	X	X	X	–
30 and 60 mins	X	X	X	X	X	X	X	X	X	X
Prior to initiation of third treatment[1]	X	X	X	X	X	X	X	X	X	X
2 mins	X	X	X	X	X	X	X	X	X	–
5 mins	X	X	X	X	X	X	X	X	X	X
10 mins	X	X	X	X	X	X	X	X	X	X
15 mins	X	X	X	X	X	X	X	X	X	–
30 and 60 mins	X	X	X	X	X	X	X	X	X	X
										0.5 mL; EDTA anticoagulant

ECG = Electrocardiogram; HR = Heart Rate; PAP = Pulmonary Arterial Pressure;
SAP = systolic arterial pressure; DAP = diastolic arterial pressure; MAP = Mean Arterial Pressure;
LVP = left ventricular pressure; dp/dt = derived from LVP; CO = Cardiac Output.

[1] Treatment was not initiated until acceptable normal baseline values were attained and accepted by the Study Director and Sponsor (if available).

Cardiac Output (CO): CO was measured by the thermal dilution method with injection of cold saline via the thermistor-tipped catheter.

Blood Pressure: SAP, DAP and MAP were measured from the implanted femoral artery catheter and recorded on the ——— Physiograph.

Pulmonary Arterial Pressure (PAP): PAP was measured from the implanted cardiac output catheter and recorded on the ——— Physiograph.

Left Ventricular Pressure (LVP): LVP and derived contractility (dp/dt) were measured from the implanted left ventricular catheter and recorded on the ——— Physiograph.

Electrocardiograms (ECG): ECG was recorded with limb leads I, II, III, aVR, aVL, and aVF. Three leads were monitored simultaneously. Results were reviewed and interpreted by a Board-certified veterinary cardiologist. The heart rate (HR) was derived from the ECG.

Blood Sample Collection: blood samples were collected from the femoral vein for hematology analysis pre dosing and 5, 10, 30, 60 minutes post dosing, as shown in Tables 2 and 3.

Confirmation of Catheter Placement: at the end of study, animals were euthanized by overdose of sodium pentobarbital followed by opening the thoracic cavity and determining correct placement of all catheters.

Table 3. Hematology Analysis

Hematology	
Total leukocyte count (WBC)	Mean corpuscular hemoglobin (MCH)
Erythrocyte count (RBC)	Mean corpuscular hemoglobin concentration (MCHC)
Hemoglobin concentration (HGB)	Platelet count (PLT)
Hematocrit value (HCT)	
Mean corpuscular volume (MCV)	

Blood smear examination (including differential)
- Relative and absolute nucleated red blood cell count (NRC, ANRC)
- Anisocytosis (ANI)
- Poikilocytosis (POI)
- Hypochromasia (HYP)
- Polychromasia (POL)
- Relative and absolute polymorphonuclear neutrophil count (PLY, APLY)
- Relative and absolute band count (BND, ABND)
- Relative and absolute lymphocyte count (LYM, ALYM)
- Relative and absolute monocyte count (MNO, AMNO)
- Relative and absolute eosinophil count (EOS, AEOS)
- Relative and absolute basophil count (BSO, ABSO)

Results

Clinical Observations: all animals recovered from the surgical procedures without significant adverse observation. The average recovery period was 60 minutes. No treatment-related clinical signs were noted.

Hemodynamics (Figure 1): the hemodynamic parameters measured at 2, 5, 10, 15, 30 and 60 minutes after each dosing (3 doses) showed great fluctuation, particularly PAP, in both saline and AF0150 groups. The variations were due to the movement of the conscious animals and position changes. PAP tended to increase in AF0150-treated animal as compared with the baseline (pre dosing).

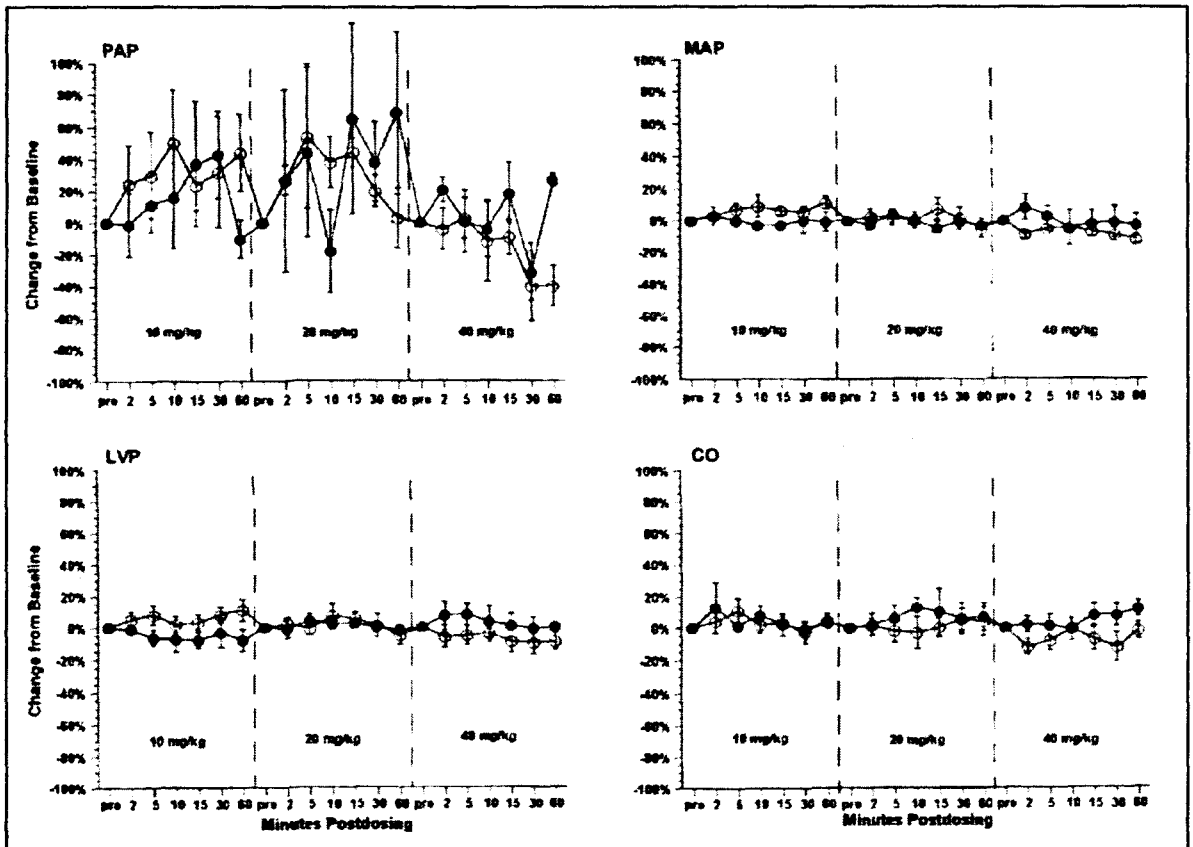

Figure 1. Percent change from baseline for pulmonary arterial pressure (PAP), mean arterial pressure (MAP), left ventricular pressure (LVP), and cardiac output (CO) following intravenous administration of Saline (○) or AF0150 (●) to conscious cynomolgus monkeys. Saline-treated animals received equivalent dose volumes to the AF0150-treated animals. Data are Mean ± SEM, n=3-4.

ECG: all animals showed normal sinus rhythm complexes with ventricular extrasystoles, without differences in the changes between saline and AF0150, and between pre and post dosing. The

ventricular extrasystoles are expected when intracardiac catheters were in place. However, no summary data and individual data were provided.

Hematology: all measured blood cells decreased throughout the entire study period in both saline and FA0105-treated animals, which may be due to the hemodilution by the multiple flushes during hemodynamic measurements.

Discussion and Comments

All hemodynamic parameters (SAP, SAP, MAP, PAP, LVP) measured at 2, 5, 10, 15, 30 and 60 minutes after each dosing (3 doses) showed great fluctuation, particularly PAP, in both saline- and AF0150-treated monkeys. It appeared that there were no differences in these parameters between AF0150 and control groups. However, with great variations it is difficulty to draw a conclusion whether AF0150 affects hemodynamic. PAP tended to increase in AF0150-treated animal as compared with the baseline (pre dosing). Arterial blood gas analysis was not observed in this study. This would have allowed an evaluation of pulmonary function and support the changes in PAP.

No remarkable findings in ECG examinations were noted. However, summary and individual EGC data were not provided in this study report. After a T-Con with the sponsor on March 30, 2000, the partial ECG data including QT, HR (heart rate), HR/QT (for QT correcting) and RR were submitted by fax on April 12, 2000. There appeared to be no significant difference in QT and "corrected" QT (HR/QT) intervals between AF0150 and saline control groups, and between pre and post dosing time points. The reviewer re-calculated QT data from pre and 30 minutes post dosing group using Bazett's formula (commonly used in human ECG). There were no significant differences in QTc between AF0150 and saline controls at 30 minutes post dose, and between pre and 30 minutes post AF0150 dosing.

There was no concurrent application of ultrasound with AF0150 administration in this study.

Report Number: IMUS-035-TOX
Safety Evaluation of AF0150 with Concurrent High Power Ultrasound Imaging in Dogs

Report Location:	Vol.023, p001-197
Report date:	October 30, 1998
Study Facility:	[redacted]
In-life phase:	January 20-21, 1998
GLP Compliance:	Yes (with QA Statement)
AF0150 Lot number:	UA16010
AF0150 Dosage (HDM):	20 mg/kg (86 fold PCD)

Specific Aim

To evaluate toxicity of AF0150 with concurrent application of high power ultrasound.

Methods

Animal Preparation: ten male Beagles were obtained from [redacted]
The animals were 7-8 months old with body weight of 9-11 kg at initiation of treatment. Standard procedures were followed for housing, handling, feeding and care of the animals. The dogs (9) were acclimated and quarantined for approximately one week before initiation of treatment, and randomly assigned into 3 groups (3/group), as seen in table 1.

Ultrasound Application and AF0150 Administration (Table 1): each animal in all groups was anesthetized with IV injection of medetomidine (40 ug/kg) through the saphenous vein, positioned for optimal cardiac imaging and the left thoracic region was shaved to expose an area approximately 10 to 15 cm2. An [redacted] ultrasound unit was used for imaging at standard cardiac setting. High power ultrasound imaging were performed for 2 minutes pre dosing, throughout the 20-minute infusion and 10 minutes after completion of infusion in Groups 1 (saline) and 2 (AF0150). AF0150 (20 mg/kg) was given to animals in Groups 2 and 3 by IV infusion (0.05 ml/kg/min) through the saphenous vein for 20 minutes. All animals received 200 ug/kg atipamezole, an antidote to the anesthesia, by IM injection following completion of saline or AF0150 infusion and imaging.

Table 1. Group assignment for safety evaluation of AF0150 combined with ultrasound application

Group #	Number of Dogs	AF0150 (mg/kg)	Ultrasound
1	3	0	Yes
2	3	20	Yes
3	3	20	No

Observation Parameters: clinical signs were observed at 1-2 and 24 hours post dosing. Body weight was recorded at pre dosing (week –1) and on the day of treatment. Blood samples were collected from the jugular vein at pre-treatment (week –1) and at 0, 20, 30, 60 minutes, 3, 6, and 24 hours post treatment for hematology, coagulation and clinical chemistry analysis. ECG was recorded in all animals from leads I, II, III, aVF, aVL and aVR once during the quarantine period, 5 minutes pre dosing (under anesthesia), 1, 5, 10, 15, 20 and 30 minutes post doing. Macroscopic examination was taken at necropsy (24 hours post treatment) and representative samples of selected organs and tissues were collected and preserved. Sections of the heart and all gross lesions were evaluated histopathologically, and all other tissues were saved for possible future examination.

Results

Clinical Signs and Body Weight: no remarkable clinical signs and body weight changes were observed in all animals.
ECG: The report from the veterinary cardiologist showed no AF0150-related changes on ECG examination. However, when reviewing the summary and individual data in the appendix of this report, QT interval slightly increased with a time-dependence in AF0150-treated dogs either with or without ultrasound application. 30% increase in QT interval was noted at 30 minutes post AF0150 dosing, as compared to pre dosing measurements. QTc was not evaluated. However, there were no corresponding changes on heart rates, although the heart rates were highly variable.

Hematology and Blood Chemistry: no remarkable observations.

Pathology: there were no macroscopic and microscopic changes associated with AF0150 treatment with or without concurrent ultrasound application.

Discussion and Comments

IV infusion of AF0150 at 20 mg/kg with concurrent application of high power ultrasound over the heart (closed-chest) had no significant effects on clinical signs, hematology, blood chemistry and pathology in dogs during the 24-hour post dosing observation period.

However, QT interval increased time-dependently in AF0150-treated dogs with and without ultrasound application, as compared to the saline control group and pre dosing measurements (under anesthesia). The QT prolongation seemed not to correspond to changes in heart rates. On March 30, 2000, we had a T-con with the sponsor and the sponsor clarified that the QT interval data in the submission was not the corrected QT. There is no particular formula for animal QT correction (as per consultation with the Division of Cardio-Renal Drugs at CDER/FDA). Therefore, the Bazett's and Fredericia's formulas, which are most commonly used in humans, were applied to calculate corrected QT (QTc). Both uncorrected and corrected QT interval data and heart rates are presented in Figure 1. It seems that there are no significant differences in the QTc intervals between AF150 and saline control groups, and between pre and post AF0150 dosing. However, the heart rates (thus R-R intervals) were very variable, which would reflect on the QTc results and makes it very difficult to draw a conclusion.

On April 12, 2000, the sponsor submitted QTc data by fax, in which Fredericia's method was used and the results were consistent with the reviewer's analysis. The sponsor concluded that there was no increase in QTc in AF0150-treated anesthetized dogs.

It is recommended that ECG examination needs to be performed in non-anesthetized animals without surgical interference and more than one dose group need to be included.

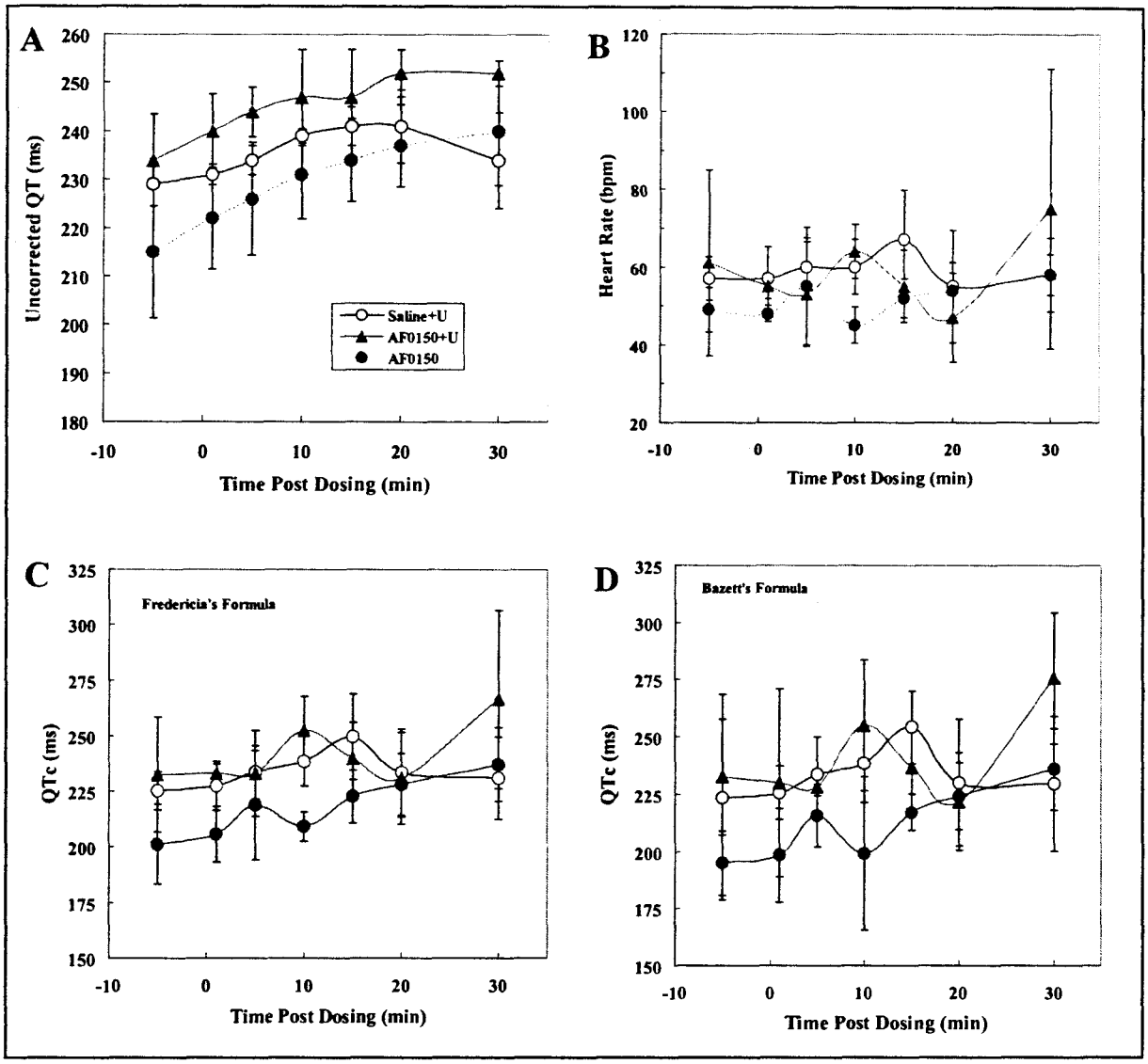

Figure 1. Effects of AF0150 on QT Interval in Anesthetized Dogs. AF0150 (20 mg/kg) or saline was given to the anesthetized dogs (male, adult) by IV infusion for 20 minutes with concurrent application of high power ultrasound over the heart (closed-chest) during the infusion period and 10 minutes following completion of the infusion. ECG was recorded 5 minutes pre dosing and up to 30 minutes post dosing. ***Panel A,*** Uncorrected QT interval; ***Panel B,*** Heart rate (converted from R-R interval); ***Panel C,*** Corrected QT (QTc) by using Fredericia's formula ($QTc=QT/(R-R)^{1/3}$); ***Panel D,*** Corrected QT interval by using Bazett's formula ($QTc=QT/(R-R)^{1/2}$). Data are mean ± SD of 3 dogs.

Report Number: PSM-98-01
Effect of Intracarotid Injection of AF0150 in Rats

Report Location:	Vol. 009, p323-323
Report date:	May 11, 1999
Study Facility:	Alliance Pharmaceutical Corp
In-life phase:	December 8-16, 1998
GLP Compliance:	No
AF0150 Lot number:	ZY18032
AF0150 Dosage (HDM):	0.125, 2, 4, 8, 16 mg/kg (0-20 fold PCD)

Specific Aim

To determine neurotoxicity of AF0150 following intracarotid injection to evaluate the potential neurotoxicity in patients with a right-to-left shunt [*instead of a full report, an abstract was provided; this study served as a pilot for the GLP study of MUS-042-TOX*]

Methods

Ten rats (SD, body weight of 367 to 420 g) were anesthetized with a ketamine/xylazine cocktail. A carotid artery was cannulated for AF0150 administration (reconstituted to a final concentration of 20 mg/ml). After a 48 hour recovery period, one rat received 0.3 m of saline as a control, and 5 rats received a single dose of AF0150 over 30 seconds. The following doses were administered: 0.125, 2.0, 4.0, 8.0 and 16.0 mg/kg. The animals were observed for gross neurological and behavioral deficits (as indicated by signs of motor or proprioceptive deficits, ataxia, paresis, paralysis, head tilt, circling or seizures) hourly for 5 to 6 hours post-dosing. After being unattended overnight, hourly observations resumed in the morning and continued up to total observation time of 24 hours post-dosing.

Results

Three of 10 rats died from surgical complications prior to administration of AF0150, and one rat was excluded from the study because the catheter was placed in the wrong vessel.

No marketed signs of motor or proprioceptive deficits, ataxia, paresis, paralysis, head tilt, circling or seizures, and no behavioral changes were noted in any of the 6 rats. All animals remained bright, alert and responsive throughout the course of the study.

Discussion and Comments

The results of this study suggest that intracarotid injections of AF0150 at doses up to 16.0 mg/kg (20-fold of PCD based on body surface area conversion) did not induce marketed neurological or behavioral effects in rats 6 and 24 hours post-dosing. However, the animals were not observed during the night period. Reversible neural effects may have been missed during that time period.

Additionally, no histological evaluation of the brain was conducted, nor were any biochemical parameters assayed.

Report Number: IMUS-042-TOX
A 7-Day Single Dose Toxicity Study of AF0150 Administrated Intra-arterial (via carotid artery) in Sprague-Dawley Rats

Report Location:	Vol.024, p001-358
Report date:	July 13, 1999
Study Facility:	[redacted]
In-life phase:	February 2-10, 1999
GLP Compliance:	Yes (with QA Statement)
AF0150 Lot number:	UA18027 (200 mg/vial)
AF0150 Dosage (HDM):	4 and 16 mg/kg (5.2-20 fold PCD)

Specific Aim

To assess the acute toxicity of AF1050 with intra-arterial injection in rats for evaluation of potential toxicity in patients with cardiovascular anomaly, such as a right-to-left shunt

Methods

Animal Preparation: Sprague-Dawley rats (40 each sex, 10-14 weeks old) were obtained from [redacted] Standard procedures were followed for housing, handling, feeding and care of the animals. After acclimation and quarantine for 7 days, animals were surgically instrumented with a catheter into the carotid artery (the tip of the catheter was in the ascending aorta). Based on catheter patency, the animals were randomly assigned into 3 groups (5/sex/group), as seen in table 1.

Table 1. Single Dose Acute Toxicity with Intra-arterial Injection in Rats

Group	AF0150 Dose* (mg/kg)	Number of Rats		IV Volume (ml/kg)
		Male	Female	
1(Control)#	0	5	5	0.8
2(Low)	4	5	5	0.2
3(High)	16	5	5	0.8

* AF0150 (200 mg fill/vial) was reconstituted in SWFI to final concentration of 20 mg/ml.
# 0.9% NaCl for Injection

AF0150 Preparation and Administration: AF0150 (200 mg fill) was reconstituted with 10 ml SWFI to a final concentration of 20 mg/kg. After recovery from the surgical procedure (4-6 days), the animals received an intra-arterial injection of saline (Group 1), 4 mg/kg AF0150 (Group 2), or 16 mg/kg AF0150 (Group 3) through the implanted carotid artery catheter. Individual doses were calculated based on the most recent body weight.

Observation Parameters:

Clinical Signs: Observation started on Day 1 prior to treatment and daily post dosing till Day 8 (necropsy), including but not limited to, changes in the skin and hair, eyes and mucous membranes, respiratory system, circulatory system, central nervous system, somatomotor activity, and behavior pattern and the occurrence of tremors, convulsions, salivation, diarrhea, or lethargy.

Functional Observational Battery (FOB): a neurobehavioral assessment designed to evaluate neurological integrity, which was performed at 5 minutes, 2, 24 hours and Day 8 (prior to necropsy) post dosing according to a standard operating procedure of "Functional Observational Battery" (included in Appendix G of the study report).

Spontaneous Locomotor Activity Test Scoring: Spontaneous locomotor activity was measured according to a draft standard operating procedure for "Spontaneous Locomotor Activity Test" (presented in Appendix H of the study report). Locomotor activity was scored in half-hour intervals from approximately 2-24 hours post dosing.

Body Weights: Individual body weights were recorded prior to surgery, pre dosing and on Days 1, 4, and 8 (necropsy).

Pathology: all animals on Day 8 were subjected to necropsy (with a routine macroscopic examination) and the location of the carotid artery catheter tip was confirmed. Histopathology examination was performed only on brain.

Results

Clinical Signs: some animals in Group 1 (saline) and Group 3 (16 mg/kg AF0150) died within the first 4 days post dosing (*Table 2*). No death was noted in Group 2 (4 mg/kg AF0150). Clinical observations showed right eye squint, head tilt to the right and partial or complete disuse of a limb and/or paw in both saline- or AF0150-treated animals pre and post dosing (*Table 2, upper panel*), particularly seen in those moribund animals. These observations were likely due to post surgical complication related to thrombus formation around the indwelling arterial catheter and subsequent dislodging of thrombi into the circulation. Other neurological signs were observed only post dosing (both saline and AF0150) which may be associated with cerebral ischemia (*Table 2, lower panel*).

Body Weight and Food Consumption: none of the animals had any remarkable changes in body weight and body weight gains related to AF0150 treatment. Food consumption was not observed.

Table 2. Clinical Observations (neurological signs)

Group Number	Gender	Found dead or moribund euthanasia	Right head tilt		Right eye squint		Disuse, partial or complete	
			Pre-dose	Post-dose	Pre-dose	Post-dose	Pre-dose	Post-dose
1	Male	1/5	4/5	3/5[a]	4/5	4/5	2/5	3/5
	Female	1/5	5/5[a]	2/5	2/5	3/5	2/5	2/5
2	Male	0/5	3/5	1/5	2/5	3/5	0/5	0/5
	Female	0/5	5/5	5/5	3/5	3/5	0/5	0/5
3	Male	2/5	3/5	1/5	4/5	4/5	0/5	0/5
	Female	0/5	5/5	4/5	3/5	3/5	2/5	2/5

[a] One animal displayed a head tilt to the left.

Group Number	Gender	Found dead or moribund euthanasia	Hunched posture		Lethargic		Ataxia	
			Pre-dose	Post-dose	Pre-dose	Post-dose	Pre-dose	Post-dose
1	Male	1/5	0/5	0/5	0/5	0/5	0/5	1/5
	Female	1/5	0/5	1/5	0/5	1/5	0/5	0/5
2	Male	0/5	0/5	0/5	0/5	2/5	0/5	0/5
	Female	0/5	0/5	0/5	0/5	0/5	0/5	1/5
3	Male	2/5	0/5	1/5	0/5	2/5	0/5	0/5
	Female	0/5	0/5	0/5	0/5	1/5	0/5	0/5

Functional Observational Battery (FOB): there were no dose- and time-dependent changes in FOB scores in AF0150-treated animals. Other than the clinical signs noted during clinical observations, FOB testing at 5 minute, 2 and 24 hours, and Day 8 post dosing revealed no consistent abnormal neurological responses.

Spontaneous Locomotor Activity (SLA): The Photobeam Activity Cage Rack system, consisting of three photobeam emitter-receiver pairs across the width of a cage, was used to count repeated activities/movements (i.e., beam interruptions). The repeated activities at the same photobeam (Beam 1, 2 or 3) were scored as fine movements, and at sequential breaks of adjacent photobeams scored as gross locomotor activity (e.g., walking) (ambulations). Animals were scored over 46 30-minute intervals for fine and gross movements, and total movements (fine movements plus ambulations). There were no significant dose- and time-dependent changes in fine and gross movements in both saline- and AF0150-treated animals.