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A non-surfactant formulation for alfaxalone based on an amorphous cyclodextrin: Activity studies in rats and dogs

K.S. Estes [1,3], M.E. Brewster [1], A.I. Webb [2] and N. Bodor [1]

[1] *College of Pharmacy and* [2] *College of Veterinary Medicine, Center for Drug Design and Delivery, University of Florida, Gainesville, FL 32610 (U.S.A.)*

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Summary

The steroid anesthetic, alfaxalone, is commercially available for veterinary use in a formulation containing Cremophor-EL® and alfadalone acetate. The allergic sensitivity of some species to this formulation limits its veterinary application and also led to withdrawal of the product for human use. The present studies evaluated alfaxalone formulated as an inclusion complex with a mixture of 2-hydroxypropyl-β-cyclodextrin (HPCD) isomers for anesthetic properties in rats and dogs. Aqueous solutions of alfaxalone-HPCD showed identical anesthetic profiles in rats compared to the commercial formulation. In contrast, the anesthetic response to alfaxalone-HPCD in dogs was not accompanied with massive histamine release and pronounced decrease in respiration and blood pressure seen after the commercial preparation. Anesthesia induction and recovery phases were rapid and uncomplicated after alfaxalone-HPCD. An anaphylactoid-like response did occur in dogs after the same dose of alfaxalone as the commercial preparation. The favorable anesthetic profile of alfaxalone formulated as an HPCD complex together with previous toxicology and stability studies suggest that the steroid may be successfully formulated for intravenous dosing with modified cyclodextrins.

Introduction

The introduction of the steroid anesthetic, alfaxalone (3α-hydroxy-5α-pregnane-11,20-dione), to both human and veterinary medicine in 1971 was favorably received and rapidly accepted by practicing clinicians (Wright and Dundee, 1982; Hall and Clarke, 1983). Developmental efforts to find a safe and effective injectable steroid anesthetic were stimulated by studies of Selye who reported in 1941 that progesterone-like steroids had anesthetic properties (Sutton, 1972). Results of this 30-year search culminated in alfaxalone which showed a therapeutic index or safety profile several times greater than that of thiopental. The anesthetic profile also indicated that rapid surgical anesthesia was induced with minimum excitation and anesthesia could be maintained for several hours with relatively rapid recovery. Rapid hepatic metabolism minimized the cumulative effects of the anesthetic and no apparent enzyme induction occurred. Additionally, no antianalgesic effects were evident (see Morgan and Whitwam, 1985).

Unfortunately, an unacceptable incidence of allergic adverse reactions, estimated between 0.1 and 0.25%, resulted in withdrawal of Althesin®,

Correspondence: K.S. Estes, P.O. Box 730, Alachua, FL 32615, U.S.A.

the alfaxalone formulation for human use (Morgan and Whitwam, 1985). Allergic adverse reactions reported in animals from the identical formulation also limit the veterinary application of the product, Saffan®. Evidence indicates that the allergic responses can be attributed to the surfactant component of the formulation, polyoxyethylenated castor oil, Cremophor-EL® (Hall and Clarke, 1983). Because dogs had a particularly high incidence of adverse reactions to Saffan and histamine response to Cremophor is pronounced in dogs, the product is contraindicated for use in this species.

In addition to Cremophor (200 mg/ml), the commercial preparation of Althesin® and Saffan® also contains the 21-acetoxy derivative of alfaxalone, alfadalone acetate (3 mg/ml), which has approximately one half the anesthetic potency of the parent steroid. Addition of alfadalone acetate permitted solubilization of alfaxalone to the 9 mg/ml level present in Saffan. Dosage of the preparation is therefore expressed as milligrams of total steroid (12 mg/ml) or as volume units (μl/kg).

The present studies were designed to evaluate an alternative formulation based on complexation of alfaxalone with amorphous cyclodextrin derivatives. This approach for solubilizing steroids was suggested by both Pitha (Pitha and Pitha, 1985) and Müller and Brauns (1985). Preliminary studies in our laboratory showed the feasibility of this approach for alfaxalone and indicated that a mixture of 2-hydroxypropyl-β-cyclodextrin isomers (HPCD) has several advantages for parenteral formulations (Brewster et al., 1989a). Solubilization with HPCD permitted a 10-fold increase in alfaxalone solubilization. No co-solubilizers were required and the solutions were stable after exposure to autoclave sterilization. The primary advantage of a cyclodextrin based parenteral formulation is elimination of the surfactant component from the current product which is potentially associated with allergic type adverse events.

The studies reported herein first demonstrate the anesthetic equivalence of an HPCD formulation with the commercial preparation in rats. Second, the histamine response of the two preparations is compared in dogs, a species shown to be especially sensitive to surface-active agents (Gaudy et al., 1987).

Materials and Methods

The steroid inclusion complex was made using micronized alfaxalone (Institute for Drug Research, Budapest, Hungary) and HPCD (Pharmatec, Inc., Alachua, FL) as previously described (Brewster et al., 1989). Briefly, an excess of alfaxalone was added to a 43.5% (w/v) solution of HPCD and the mixture was sonicated to facilitate incorporation. The mixture was then filtered, and the filtrate was then frozen in liquid nitrogen and lyophilized (Labconco Freeze Dryer Model 18). The dried complex was sieved (60 mesh) and the incorporation of alfaxalone was determined using HPLC. The extent of incorporation for material used in the present studies was 83 mg alfaxalone per g powder. Additional HPCD was mixed with the powdered Alf-CD complex to yield 60 mg and 45 mg alfaxalone/g powder for rats and dogs, respectively, before adding water. Test solutions (Alf-CD) containing alfaxalone in 20% (w/v) HPCD were prepared in deionized water (Barnstead Nanopure II Ultrapure Water System) daily. Studies conducted in rats used Alf-CD solutions containing 12 mg/ml of steroid while the study in dogs used a 9 mg/ml steroid solution. Saffan (Glaxo, Research Triangle Park, NC) was used as kindly provided by the manufacturer.

Sprague Dawley male (445–540 g) and female (230–385 g) rats (Charles Rivers, Wilmington, MA) were individually housed in a temperature ($22 \pm 2°C$) and light (14 h light) controlled vivarium and provided with free access to Purina Laboratory Chow and tap water. Animals were subjected to four testing sessions. Each session monitored several parameters. Duration of loss of righting response after drug administration was recorded as an index of sleeping time. The degree of anesthesia during sleeping was estimated by the presence or absence of a visible response to auditory stimulation (hand clap 10 cm over the supine animal) and response to abdominal pinch stimulation (tissue forceps pinch) was recorded at 5–10-min intervals. The time (s) required for the tail

flick response after submerging the tail in water maintained at 55–58 °C was recorded before drug and 15 min after regaining righting response. This was used to evaluate neuromuscular response and as an index of analgesic or antianalgesic activity. Saffan and Alf-CD test solutions were administered via tail vein injection (0.25, 0.5 or 1.0 ml/kg) and by intraperitoneal injection (2, 4 or 6 [females] or 8 [males] ml/kg).

Six mongrel dogs (20–32 kg) were conditioned and maintained under standard kennel procedures (Department of Animal Resources, University of Florida). All experimental procedures were conducted in compliance with the Institutional Animal Care and Use Committee recommendations. Dogs were randomly assigned to treatment with either Alf-CD (0.75 ml/kg, $n = 4$) or Saffan (0.75 ml/kg, $n = 2$) solutions administered at 20 ml/min through an i.v. catheter. Blood samples were obtained from the jugular vein prior to test drug administration, at 15 min after induction of anesthesia, and 24 h after drug dosing. Routine clinical chemistry profiles were characterized from pre-dose and 24 h post-dose samples. Histamine levels in the pre-dose and 15 min plasma samples were determined by radioenzymatic assay methods (Faraj et al., 1984, 1986). Indirect systolic blood pressures, heart rates and respiratory rates were frequently recorded during the 30 min post-drug interval. The antihistamine, diphenhydramine (30 mg), was administered 30 min following Saffan treatment. The times to extubation and return to ambulation were recorded.

Results

The anesthetic profile of alfaxalone formulated as a 20% (w/v) HPCD solution was very similar to that of the commercial Cremophor formulation in rats. As shown in Fig. 1, sleeping duration was identical after i.v. dosing (top panel). Linear regression analysis followed by t-tests for homogenicity of regression showed no significant differences between treatment groups (Steel and Torrie, 1960). Further, the response to abdominal pinch was absent at 1 and 5 min and returned at 10 min post injection with the largest dose of both preparations. Tail flick response was not different with i.v. treated animals at 15 min after return of the righting response (range 3–8 s) compared to pre-dose values. After i.p. dosing, a sex difference was found in sleeping times for both Saffan and Alf-CD which was statistically significant at $P < 0.001$ for both compounds (Fig. 1, bottom panel). A sex difference was previously reported after i.p. dosing with Saffan with females observed to be more sensitive to the anesthetic (Fink et al., 1982). The slopes of sleep duration vs total steroid dose were significantly different between i.p. Alf-CD- and Saffan-treated groups for both males and females ($P < 0.01$). However, when the reduced biological potency of alfadalone acetate was con-

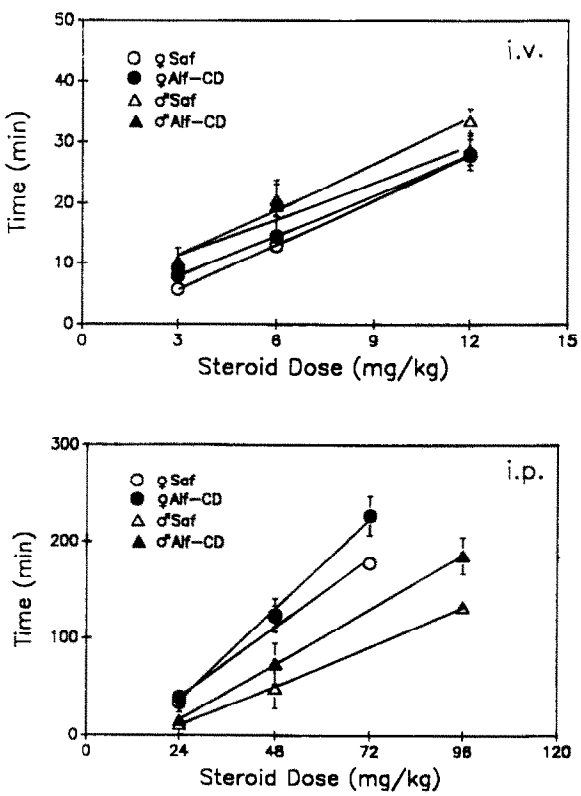

Fig. 1. Dose-response profiles for alfaxalone formulation, Alf-CD (12 mg/ml) and Saffan (Saf) in male and female Sprague Dawley rats. (Top panel) Comparison of sleeping times monitored as duration of loss of righting response after intravenous drug administration. (Bottom panel) Sleeping times in the same groups of male and female rats dosed via intraperitoneal injection (i.p.). Vertical bars represent S.E.; $n = 3$–4 rats/group.

sidered and data were expressed as alfaxalone potency vs sleep duration no significant difference between preparations was detected. Sex differences remained statistically significant after correcting for alfadalone acetate ($P < 0.001$) in i.p. treated groups. The duration of absent pinch response was more variable within groups after i.p. dosing (range 15–195 min for both formulations). Response to auditory stimulation was usually coincident with or appeared rapidly after the positive response to pinch stimulus. Excitation was not apparent during induction of anesthesia

TABLE 1

Measured parameters in dogs after Alf-CD or Saffan

	Drug	Alf-CD	Alf-CD	Alf-CD	Alf-CD	Saffan	Saffan
	Dog No.	A	B	C	F	D	E
	Weight (kg)	29.3	31.3	26.7	29.3	32.4	20.7
	Dose (ml)	22.0	23.5	20.0	22.0	24.3	15.5
Sedation (min)							
extubation		35	18	18	37	100	105
standing		42	45	43	40	165	200
Histamine (ng/ml)							
pre-dose		0.09	0.08	3.64	5.53	5.60	7.41
post-dose (15 min)		1.15	4.62	3.66	7.80	88.43	90.13
increase		1.06	4.54	0.02	2.27	82.83	82.72
Heart rate (beats/min)							
pre-dose		120	140	100	156	92	100
1 (min)		180	140	156	168	164	164
2		162	140	152	174	186	156
5		162	128	156	135	208	144
10		144	108	140	120	204	164
15		132	100	130	126	208	148
20			100	138	128	198	162
25					118	180	136
30					132	167	152
Respiration (beats/min)							
1 (min)		12		16	16	12	IPPV
2			16	8		IPPV [a]	IPPV
5		12	12	12	16	IPPV	IPPV
10		20	16	36	16	IPPV	IPPV
15		20	32	64	18	IPPV	IPPV
20					22	IPPV	IPPV
25					28	IPPV	
30					18		
Blood pressure (mmHg)							
1 (min)		140		130		N.D. [b]	N.D.
2			174		38	N.D.	N.D.
5		120	140	108	48	N.D.	N.D.
10		120	130	100	64	N.D.	N.D.
15		118	140	105	98	N.D.	N.D.
20				115	106	N.D.	N.D.
25					112	N.D.	N.D.
30		125			110	N.D.	N.D.

[a] IPPV, intermittent positive pressure ventilation.

[b] N.D. not determined (< 35 mmHg).

for any group. No tissue irritation was observed at the injection site in any treated rat.

In contrast to the similar response observed in rats following treatment with Alf-CD or the commercial preparations, dogs showed clearly different response to the formulations. Heart rate, respiration rate and indirect blood pressure data for the six individual animals are listed in Table 1 to demonstrate the variation in response seen within and between groups. Both dogs treated with Saffan had an anaphylactoid type response characterized by a rapid and pronounced fall in blood pressure to levels which could not accurately be monitored with the external monitoring apparatus used. In contrast, blood pressures were relatively stable for the Alf-CD-treated dogs, except in one animal which had a pronounced but transient decrease in pressure. The anaphylactoid response after Saffan treatment precluded study of additional animals for a statistically balanced study design. Saffan-treated dogs required manual ventilation while other animals maintained spontaneous respiration. The lungs were markedly noncompliant during manual ventilation which is consistent with histamine-induced pulmonary edema. As shown in Fig. 2, there was a pronounced elevation in plasma histamine 15 min following Saffan (82.8 ng/ml) compared to the difference measured after Alf-CD (2.0 ± 1.0 ng/ml). Heart rate increased after drug administration for all animals except one dog in the Alf-CD group. Elevated heart rate appeared more pronounced and sustained in both Saffan-treated dogs compared to Alf-CD animals (Table 1). Diphenhydramine was administered 30 min after Saffan to block histamine effects at H_1 receptors. All dogs recovered, however sedation was 3–5-times longer in Saffan-antihistamine-treated dogs as compared to Alf-CD (Table 1).

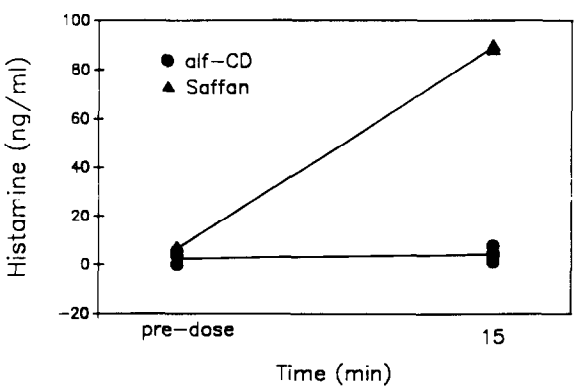

Fig. 2. Plasma histamine response in mongrel dogs immediately before (pre-dose) and 15 min after dosing with Saffan or Alf-CD (9 mg/kg, i.v.). Each symbol represents an individual animal. Regression lines are shown connecting the mean group values.

Discussion

The results from these in vivo studies clearly show that the steroid anesthetic, alfaxalone, formulated as a modified cyclodextrin inclusion complex, maintains an anesthetic profile comparable to the Cremophor containing commercial formulation, Saffan.

The anesthetic effect observed after i.v. dosing was rapid, requiring only a few seconds in both rats and dogs. These results are consistent with a nearly instantaneous release of steroid from the cyclodextrin complex after administration. Our previous studies examined the stoichiometry of the complex and the phase solubility profile of alfaxalone in HPCD (Brewster et al., 1989a). It was shown that while the amorphous complex has a molar ratio of 1 : 1.7 (83 mg/g) for alfaxalone : HPCD moieties, the phase solubility profiles are in support of a 1 : 1 molar ratio being present for the complex in solution.

In the presently reported studies, the anesthetic profile was identical in rats after i.v. dosing with either Alf-CD or Saffan. A sex difference was evident for both formulations after i.p. administration with female rats being more sensitive to the anesthetic effects of alfaxalone than males. This sex difference was previously observed and attributed to an estrogen potentiation of the anesthetic effects (Fink et al., 1982). More recent studies have implicated 3α-hydroxydihydroprogesterone as an endogenous modulator of the GABA receptor chloride channel complex. This mechanism appears to be responsible for the anesthetic activity of alfaxalone (Turner et al., 1989). Sex differences in concentration of this progesterone metabolite may explain the increased

sensitivity to alfaxalone observed after i.p. dosing in females. Failure to detect sex differences after i.v. dosing in the present studies may be associated with the short duration of drug action using this administration route.

A marked difference was noted between dogs anesthetized with the two alfaxalone preparations. The two animals treated with Saffan had an immediate and pronounced anaphylactoid-type response characterized by drop in blood pressure and respiration with a striking increase in plasma histamine. In contrast, anesthesia with 9 mg/kg of the Alf-CD preparations was uneventful and characterized by smooth induction and recovery profiles. Some cardiovascular change may have been effected by the anesthetic as there was a transient decrease in blood pressure in the last dog treated in the study. However, data for this parameter were not monitored continuously and the baseline pressure immediately prior to administration was not determined. An explanation for the transient (< 10 min) decrease in blood pressure may also be the possibility of residual Saffan in the infusion tubing. Heart rates were transiently increased in three of four Alf-CD-treated dogs and markedly increased for the 30 min monitoring period in both Saffan-treated dogs. Additional studies in a variety of animal models are required to identify the nature of potential cardiovascular effects of this Alf-CD formulation.

The dog model is known to be particularly sensitive to the Cremophor component in the commercial anesthetic preparation (Lorenz et al., 1977). Therefore, the product is contraindicated in all canine species. While the Cremophor component has been implicated as primarily responsible for the adverse allergic response reported in humans and other species, this relationship has not been directly tested in the clinic. Laboratory studies have suggested that the allergic potential of Saffan (or Althesin) is greater than that of any individual component in the preparation (Tachon et al., 1983). In studies using a guinea pig model, however, the magnitude of increased allergic response to Althesin vs Cremophor was small compared to the difference between alfaxalone in sesame oil and Cremophor. No allergic sensitizing potential has been reported for alfaxalone alone or HPCD. While it is extremely unlikely that the Alf-CD complex is sensitizing, its allergic potential should be examined in a variety of animal models.

The favorable anesthetic profile for alfaxalone demonstrated through extensive clinical experience with Althesin prior to withdrawal from the European market is supportive of developing a new formulation for the steroid. Results of solubilization and stability studies previously reported with the modified cyclodextrin, HPCD (Brewster et al., 1989) together with the maintained favorable anesthetic profile shown in the present studies and toxicological profile of the excipient (Pitha and Pitha, 1985; Pitha et al., 1988; Brewster et al., 1989a,b, 1990) are indicative of the possible benefits to be gained by further examination of an i.v. formulation for alfaxalone which uses HPCD.

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