Alphaxalone Reformulated: A Water-Soluble Intravenous Anesthetic Preparation in Sulfobutyl-Ether-β-Cyclodextrin

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BACKGROUND: Alphaxalone is a neuroactive steroid anesthetic that is poorly water soluble. It was formulated in 1972 as Althesin® using Cremophor® EL, a nonionic surfactant additive. The product was a versatile short-acting IV anesthetic used in clinical practice in many countries from 1972 to 1984. It was withdrawn from clinical practice because of hypersensitivity to Cremophor EL. In the investigations reported here, we compared the properties of 3 anesthetics: a new aqueous solution of alphaxalone dissolved in 7-sulfobutyl-ether-β-cyclodextrin (SBECD, a water-soluble molecule with a lipophilic cavity that enables drug solubilization in water); a Cremophor EL preparation of alphaxalone; and propofol.

METHODS: Two solutions of alphaxalone (10 mg/mL) were prepared: one using 13% w/v solution of SBECD in 0.9% saline (PHAX) and the other a solution of alphaxalone prepared as described in the literature using 20% Cremophor EL (ALTH). A solution of propofol (10 mg/mL; PROP) in 10% v/v soya bean oil emulsion was used as a comparator anesthetic. Jugular IV catheters were implanted in male Wistar rats (180–220 g) under halothane anesthesia. Separate groups of 10 implanted rats each were given IV injections of PHAX, ALTH, or PROP from 1.2 mg/kg to lethal doses. Doses of each drug that caused anesthesia (loss of righting reflex and response to tail pinch) and lethality in 50% of rats were calculated by probit analysis. The drugs were also compared for effects on arterial blood pressure and heart rate.

RESULTS: IV PHAX, ALTH, and PROP caused dose-related sedation and anesthesia, with 50% effective dose (ED50) values for loss of righting reflex being 2.8, 3.0, and 4.6 mg/kg, respectively. PROP led to death in 10 of 10 rats at doses >30 mg/kg (50% lethal dose (LD50) = 27.7 mg/kg). A dose of alphaxalone 53 mg/kg as ALTH caused 10 of 10 rats to die (LD50 = 43.6 mg/kg), whereas none died when given the same doses of alphaxalone formulated in SBECD. PHAX caused 20% lethality at the maximal dose tested of 84 mg/kg. PHAX caused less cardiovascular depression than PROP. Control experiments with the 3 drug-free vehicles showed no effects.

CONCLUSIONS: Alphaxalone caused fast-onset anesthesia at the same dose for both formulations (PHAX and ALTH). The use of SBECD as a drug-solubilizing excipient did not alter the anesthetic effect of alphaxalone, but it did increase the therapeutic index of alphaxalone in PHAX compared with ALTH. PHAX has a higher safety margin than the propofol lipid formulation and also the alphaxalone formulation in Cremophor EL (ALTH). (Anesth Analg 2015;120:1025–31)
developed using hydroxypropyl-β-cyclodextrin (HPBCD) as the drug-solubilizing excipient. This is marketed as Alfaxan CD-RTU (ALF).4-6 Dissolution of alphaxalone in an aqueous vehicle suitable for human use has proved to be difficult. However, 7-sulfobutyl-ether-β-cyclodextrin (SBECD) has been used to dissolve hydrophobic drugs in water for IV injections suitable for human use,7-11 but has not been investigated previously as an excipient to enable a human product of alphaxalone free from Cremophor EL.

The studies reported here compare the anesthetic properties and TIs of 3 anesthetic preparations: propofol in the form of an injectable soya bean oil emulsion, and 2 preparations of alphaxalone, one using Cremophor EL and the other using SBECD as excipients.

METHODS
This research was approved by Monash Medical Centre Animal Ethics Committee B (approval number MMC-B 2010/04). The present study was in compliance with the guidelines and recommendations from the Code of Practice for the Use of Animals From Municipal Pounds in Scientific Procedures established by the Bureau of Animal Welfare of Victoria, Australia. All rats were housed in plastic boxes at room temperature with food and water available ad libitum. A 12:12 light/dark cycle with lights on at 8:00 AM was maintained, and the tests were performed between 9:00 AM and 7:00 PM.

Drug Preparations
The propofol used in these studies was Diprivan® (propofol 10 mg/mL in 10% soya bean oil emulsion; AstraZeneca, North Ryde, NSW, Australia). Alphaxalone and alphadolone were purchased from Steraloids Inc., Newport, RI. SBECD was obtained as Captisol® from Ligand Pharmaceuticals (formerly Cydex Pharmaceuticals), Shawnee Mission, KS. Alphaxalone was prepared as ALTH as described previously: alphaxalone 9 mg/mL, plus alphadolone 3 mg/mL in 20% w/v Cremophor EL.12 Because alphadolone is reported to have the same anesthetic properties as alphaxalone but with 30% to 50% of the potency,12 for comparison with the other drugs, ALTH will be considered alphaxalone 10 mg/mL. The third anesthetic preparation, PHAX, was also alphaxalone 10 mg/mL, prepared as follows: alphaxalone 300 mg (0.9 mmol), SBECD 3889 mg (1.8 mmol) dissolved in 30 mL 0.9% sodium chloride solution. The method used was first dissolution of the SBECD in the final volume of saline by stirring and then adding the alphaxalone while continuing to stir. No heating or ultrasonication was necessary to dissolve the alphaxalone in this solution.

Rat Experiments
Anesthesia
Male Wistar rats (180–220 g weight; Monash University Animal Services) were implanted with internal jugular IV catheters under halothane anesthesia. The catheters were tunneled under the skin and brought out to the dorsum of the neck, where they were additionally anchored with nylon sutures. Twenty-four hours later, each rat was placed briefly in a Plexiglass restrainer to facilitate an IV injection over 15 seconds of a range of doses of PROP, ALTH, or PHAX (1.2–15 mg/kg). The following were assessed at regular time intervals after the IV injection:

1. The presence of a normal righting reflex, scored as present or absent,
2. Any response to tail pinch by a 25-cm artery clamp applied to the base of the tail to a maximal force of touching the first ratchet, scored present or absent, and
3. Time (seconds) that the rat was able to walk on a rotarod (a rotating cylinder; Ugo Basile Srl, Comerio, Italy): the normal run time is 120 seconds in nonsedated rats. Time from drug injection to attaining this value was a measure of time taken to recover from the sedating effects of the anesthetics.

Cardiovascular Effects
Male Wistar rats (180–220 g weight) were implanted with internal jugular vein catheters as above. Twenty-four hours later, groups of 10 rats received an IV injection from a range of doses of PROP (6.6 mg/kg), ALTH (3.3 mg/kg), or PHAX (3.3 mg/kg) being the ED95 dose of each drug for loss of righting reflex shown in Figure 1. Systolic and diastolic blood pressure and heart rate were measured using a noninvasive tail cuff blood pressure recorder with piezo-ceramic pulse detection (BP Recorders series 58000; Ugo Basile Srl). These variables were measured every 5 minutes for 15 minutes before and at 0.5, 1, 2, 3, 4, 5, 7.5, 10, 15, and 20 minutes after the IV drug treatments.

Lethal Dose
Male Wistar rats (180–220 g weight) were implanted with internal jugular vein catheters as above. Twenty-four hours later, groups of 10 rats received an IV injection from a range

![Figure 1](https://www.anesthesia-analgesia.org)  Probit plots (A, PROP; B, ALTH; C, PHAX) for loss of righting reflex and response to tail pinch. These plots were used to calculate the ED50 values to compare potencies of the anesthetic preparations shown in Table 1. Data for loss of righting reflex are shown as blue symbols (squares) and the probit regression by blue lines (solid for mean and dotted for ±95% confidence interval [CI]). Data for loss of tail pinch response are shown as red symbols (circles) and the probit regression by red lines (solid for mean and dotted for ±95% CI).
of doses of PHAX, ALTH, or PROP, with each member of a group of 10 receiving the same formulation and dose of drug. The dose of anesthetic drug injected was increased in subsequent groups of 10 until all rats in the group died. The number of rats that died was recorded for each group.

**Effect of SBECD on Lethality**
To test whether the low toxicity of alphaxalone as PHAX was attributable to the SBECD excipient, 20 rats with indwelling jugular IV catheters were divided into 2 groups of 10 rats each. They were all given IV injections of ALTH at a dose that had, in previous experiments reported above, caused all rats to die (alphaxalone dose 52.5 mg/kg IV). Sixty seconds before the ALTH injection, a premedication IV injection was given:
1. Group 1 (10 rats) received 5.3 mL/kg 0.9% sodium chloride solution.
2. Group 2 (10 rats) received 5.3 mL/kg 13% solution of SBECD in 0.9% sodium chloride solution. This dose of SBECD was chosen because this was the amount of SBECD administered when 52.5 mg/kg alphaxalone was given as PHAX.

The number of rats that died in each group of 10 after the subsequent ALTH injection was recorded.

**Controls**
Indwelling jugular IV catheters were implanted for control experiments in 15 rats. Five rats were given a 20% solution of Cremophor EL, another 5, 13% SBECD, and 5 more, 10% soya bean oil emulsion, with all 3 groups being given a dose and volume equal to that administered in the experiments above at the highest doses of alphaxalone (for ALTH, PHAX) and propofol.

**Statistics**
The number of rats in each group for the experiments above was chosen before the experiments were performed, and no changes to those planned numbers of experiments were made once the series of experiments was started. For most experiments, it was decided to use 10 rats in each comparator group because this is the number most used in experiments of this type reported in the literature using probit regression analysis to compare anesthetic potencies. For the experiments on cardiovascular effects of the 3 anesthetic preparations, n = 5 per group was chosen because this is the number used successfully in recent studies comparing cardiovascular effects of different anesthetics in rats.

The number of rats in each group of 10 similarly treated animals that lost righting reflex or did not respond to tail pinch was subjected to probit regression analysis using SPSS Statistics 18 to produce a probit plot to calculate the estimated dose that caused death in 50% of subjects (LD₅₀). The TI was then calculated for each drug: TI = LD₅₀ + ED₅₀.

The results from the experiments investigating the effect on lethality of previous administration of SBECD were entered into a contingency table and compared statistically using Fisher exact test.

**RESULTS**

**Anesthesia**
All 3 anesthetic preparations caused dose-related effects on loss of righting reflex and tail pinch response that occurred within 15 seconds of drug injection. There were no involuntary muscle movements observed with any drug. Figure 1 shows the probit plots for righting reflex and tail pinch responses of rats treated with PROP (Fig. 1A), ALTH (Fig. 1B), and PHAX (Fig. 1C). The alphaxalone formulations, PHAX (SBECD-enabled), and ALTH (Cremophor EL-enabled) were equipotent for the dose required for loss of righting reflex (ED₉₀ = 2.8, 2.2–4.3 mg/kg, and 3, 2.4–4.5 mg/kg; mean, 95% CI for PHAX and ALTH, respectively), and furthermore, both were more potent than PROP for this end point (ED₉₀ for PROP = 4.6, 3.8–5.0 mg/kg; mean, 95% CI) (Table 1).

Figure 2 shows the mean walking time on the rotarod for groups of 10 rats at each dose of the 3 anesthetic preparations: PROP (Fig. 2A), ALTH (Fig. 2B), and PHAX (Fig. 2C). It can be seen from these that recovery from sedation to a normal 120-second score in this test occurred similarly for all 3 drugs at the minimal anesthetic dose (5 mg/kg of alphaxalone as ALTH and PHAX and 10 mg/kg PROP; Table 1).

**Cardiovascular Effects**
Figure 3 shows the cardiovascular effects of the ED₉₀ dose for loss of righting reflex for each anesthetic: PROP (Fig. 3A), ALTH (Fig. 3B), and PHAX (Fig. 3C). These are
The doses of alphaxalone given as ALTH and PHAX for loss of righting reflex and tail pinch response are not statistically different, but the LD_{50} dose for alphaxalone as PHAX was greater than alphaxalone as ALTH. The duration of loss of righting reflex was not statistically significantly different between treatments at the minimal doses of each anesthetic that caused 10 of 10 rats to lose the righting reflex. The time to complete recovery to normal rotarod running times was statistically significantly less after PROP compared with ALTH but no different for PROP versus PHAX or ALTH versus PHAX.

### Table 1. Doses Causing Loss of Righting Reflex and Tail Pinch Responses and Lethality Plus Recovery Rates for 2 Alphaxalone Preparations (PHAX and ALTH) and Propofol (PROP)

<table>
<thead>
<tr>
<th></th>
<th>ALTH</th>
<th>PHAX</th>
<th>PROP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal anesthetic dose causing all rats in a group of 10 to lose righting reflex, mg/kg</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>ED_{50} dose for loss of righting reflex, mg/kg mean (95% CI)</td>
<td>3.0 (2.4–4.5)</td>
<td>2.8 (2.2–4.3)</td>
<td>4.6 (3.8–5)</td>
</tr>
<tr>
<td>LD_{50} dose for loss of tail pinch response, mg/kg mean (95% CI)</td>
<td>6.5 (4.7–8.4)</td>
<td>6.6 (5–8.3)</td>
<td>8.4 (6.7–10.4)</td>
</tr>
<tr>
<td>Duration of loss of righting reflex after minimal anesthetic dose that caused all 10 rats to lose righting reflex, minutes mean (95% CI)</td>
<td>43.6 (40.7–46.6)</td>
<td>&gt;84</td>
<td>27.7 (26.3–29)</td>
</tr>
<tr>
<td>Time (minutes) to complete recovery of rotarod Performance after minimal anesthetic dose (all 10 rats lost righting reflex), mean (95% CI)</td>
<td>19.9 (17.1–22.7)</td>
<td>17 (15.4–18.5)</td>
<td>16 (14.6–17.3)</td>
</tr>
</tbody>
</table>

CI = confidence interval; ED_{50} = 50% effective dose; LD_{50} = 50% lethal dose.

\(^{a}P = 0.3594\) 1-way analysis of variance (ANOVA); analysis of residuals revealed they were normally distributed with equal variances between treatment groups (Bartlett statistic [corrected] = 2.658; \(P = 0.2658\)).

\(^{b}P = 0.0123\) 1-way ANOVA. Tukey corrected CIs: ALTH versus PHAX, \(-0.2137\) to 6.014; ALTH versus PROP, \(-7.014\) to \(-0.7863\); PHAX versus PROP, \(-4.114\) to 2.114. Analysis of residuals revealed they were normally distributed with equal variances between treatment groups (Bartlett statistic [corrected] = 5.570; \(P = 0.0617\)).

### Lethal Dose

The probit plots for lethality are shown in Figure 4. The probit plots for ALTH and PROP were used to calculate the dose of drug that caused death in 50% of the cohort (the LD_{50}) (Table 1). Dose escalation of PROP to doses >30 mg/kg caused death in all 10 rats in the group. ALTH caused dose-related mortality. A dose of 52 mg/kg alphaxalone as ALTH caused death in all 10 rats in the group. However, 52 mg/kg alphaxalone as PHAX did not cause any rats to die in that group of 10 rats. Alphaxalone as PHAX at 71, 78, and then 84 mg/kg caused 20% mortality. The dose escalation was stopped at that point because the volume of injectate exceeded 10% blood volume. Thus, the LD_{50} of alphaxalone as PHAX is >84 mg/kg. The TI (LD_{50} + ED_{50}) is 14.8 for ALTH and >30 for PHAX, both being much higher than the TI for PROP (6.5; Table 1).

### Effect of SBEC on Lethality

All 20 rats were anesthetized by injection of 52.5 mg/kg alphaxalone as ALTH. The lethality results are shown in Table 3. In Group 1, which had received saline only as a premedication before ALTH injection, 8 of the 10 rats died. However, in Group 2, which received SBEC premedication, 2 of the 10 rats died. This reduction in mortality associated with SBEC premedication was statistically significant (\(P = 0.0230\); Fisher exact test).

### Controls

None of the 3 vehicles caused sedation, anesthesia, or death in any rat when given alone at the highest doses and volumes used in the experiments above.

### DISCUSSION

The main conclusions from this preclinical study in rats are:

- The new water-soluble preparation of alphaxalone, PHAX, causes anesthesia with fast onset and offset timing equivalent to PROP.
- The 2 alphaxalone solutions, PHAX and ALTH, are equipotent with the same ED_{50} values for loss of righting reflex and for the dose needed to abolish the tail pinch response.
The 2 alphaxalone solutions, PHAX and ALTH, have the same recovery times for sedation after the same doses of alphaxalone, as revealed by the rotarod test. PHAX has a higher TI than PROP or ALTH.

Neuroactive steroid anesthetics are characterized by high TIs, with that for alphaxalone commonly quoted at 20 for rodents. The experiments in rats reported here show that PHAX is less toxic than the propofol lipid formulation PROP, and that it causes less cardiovascular depression. It is interesting to note that PHAX is also less toxic than the alphaxalone formulation in Cremophor EL (ALTH), with TI being >30 for PHAX, and 14.8 and 6.0 for ALTH and PROP, respectively (Table 1). None of the 3 drug-free vehicles caused sedation, anesthesia, or death, indicating that neither the differences between the sedating and anesthetic properties nor the safety or lethality of the formulations of alphaxalone or propofol were attributable to dose-related toxicity of the excipients.

In the experiments testing lethality, escalating doses of the drugs were given IV at the same rate (15 seconds) into the jugular vein. No cardiovascular or respiratory support was given, so the deaths could have been a result of cardiovascular or respiratory embarrassment or a combination of both. Clinical sedating and anesthetic doses of propofol cause depression of arterial blood pressure and respiration. Furthermore, it has been reported that alphaxalone as

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**Table 2. Results of Statistical Analysis of Cardiovascular Measurements After ED₉₅ Doses of PHAX, ALTH, and PROP**

<table>
<thead>
<tr>
<th>Cardiovascular parameters</th>
<th>PROP versus ALTH</th>
<th>PROP versus PHAX</th>
<th>ALTH versus PHAX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td>0.1015</td>
<td>0.1687</td>
<td>0.0175</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.0092</td>
<td>0.0710</td>
<td>0.0267</td>
</tr>
<tr>
<td>Heart rate</td>
<td>0.5824</td>
<td>&gt;0.9999</td>
<td>0.9666</td>
</tr>
</tbody>
</table>

PROP treatment caused greater decreases in systolic and diastolic blood pressures than PHAX. There were no statistically significant differences between treatments for effects on heart rate. There were no statistically significant differences between PHAX and ALTH for effects on systolic or diastolic blood pressure. P values shown are exact.

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**Table 3. Effect of Previous Administration of SBECD on the Lethality of ALTH**

<table>
<thead>
<tr>
<th>Premedication (5.25 mL/kg IV)</th>
<th>Number of rats that died</th>
<th>Number of rats that lived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1, saline</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Group 2, 13% SBECD</td>
<td>8*</td>
<td>8</td>
</tr>
</tbody>
</table>

*P = 0.0230; Fisher exact test.
ALTH or ALF, caused minimal cardiovascular effects at clinical and supraclinical doses, so the wider margin of safety between therapeutic and lethal doses of PHAX compared with PROP was to be expected.

Alphaxalone has been successfully formulated in HPBCD as a veterinary anesthetic (ALF), and it is used widely in veterinary practice, being reported to have a high safety margin and low cardiovascular toxicity.6,15-20 The data in these papers indicate that the HPBCD formulation is well tolerated in many species, and complement activation and histamine release are not features of this formulation, compared with the veterinary equivalent of ALTH, Saffan® which contained Cremophor EL. Clearly, one has to ask the question “why not use this in humans?” The answer is one of relative toxicity for HPBCD versus SBECD.

In early development, IV voriconazole was formulated with HPBCD. The clinical voriconazole formulation that was eventually released used SBECD in the formulation instead of HPBCD because of differences in toxicity seen in animal models.21 These include hemolysis, the occurrence of foamy macrophages, and pancreatic cancers with HPBCD not seen with SBECD.22,23 Many of these effects are attributable to membrane destabilization through the removal of lipids with HPBCD, which has not been seen with SBECD.

It was unclear why PHAX should have a TI >30, when 20 was reported in the literature for ALTH in rodents. PHAX is clearly different from the veterinary formulation of alphaxalone in HPBCD, ALF, for which the LD₅₀ is stated in the product insert to be 19 mg/kg IV in rats (TI = 7.6). The explanation for these differences is the presence of SBECD in PHAX. The series of experiments in which SBECD and saline were given as a premedication before a lethal dose of ALTH showed clearly that the SBECD was responsible for the higher TI of PHAX compared with ALTH.

The use of SBECD as excipient to aid dissolution of alphaxalone avoids the major impediment to reintroduction of this useful agent into human anesthetic practice, that is, hypersensitivity reactions to Cremophor EL; the excipient used to dissolve alphaxalone as ALTH marketed as a human and veterinary anesthetic from 1972 to 1984. ALTH was used in every area of anesthetic practice, including prolonged intensive care sedation and the emerging areas of day case anesthesia and procedural sedation. It was described as very close to the “ideal” IV anesthetic, having rapid onset and offset of action, free from irritating effects on blood vessels, and causing minor cardiovascular and respiratory depression with a wide TI.3 ALTH was only withdrawn because of Cremophor EL hypersensitivity. No other safety or efficacy issues were identified. Since PHAX contains no Cremophor EL and has similar onset and offset of sedation and anesthetic action as ALTH at the same dose, it seems logical to suggest that this preparation might allow the reintroduction of alphaxalone into clinical anesthetic and intensive care practice, provided the results of the preclinical experiments reported here are reproduced in human studies.

REFERENCES


DISCLOSURES

Name: Colin S. Goodchild, MA, MB, BChir, PhD, FRCA, FANZCA, FFPMANZCA.

Contribution: This author helped design the study, conduct the study, analyze the data, and write the manuscript.

Attestation: Colin S. Goodchild has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.

Conflicts of Interest: Colin S. Goodchild has equity interest in Drawbridge Pharmaceuticals. He is cited as inventor on Phaxan patents and is also a director and owner of Drawbridge Pharmaceuticals, to which the Phaxan patents have been assigned.

Name: Juliet M. Serrao, MB, BS, PhD, FRCA.

Contribution: This author helped analyze the data and write the manuscript.

Attestation: Juliet M. Serrao has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

Conflicts of Interest: Juliet M. Serrao has equity interest in Drawbridge Pharmaceuticals. She is cited on Phaxan patents as inventor, and she is a director and owner of Drawbridge Pharmaceuticals, to which the Phaxan patents have been assigned.

Name: Anton Kolosov, MSc, PhD.

Contribution: This author helped conduct the study and write the manuscript.

Attestation: Anton Kolosov has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

Conflicts of Interest: The author has no conflicts of interest to declare.

Name: Ben J. Boyd, PhD.

Contribution: This author helped conduct the study and write the manuscript.

Attestation: Ben J. Boyd has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

Conflicts of Interest: Ben J. Boyd will receive royalties from Monash University if Phaxan is successfully commercialized by Drawbridge Pharmaceuticals. He is cited on the Phaxan patents as inventor, and as an employee of Monash University, he will receive a share of the royalties that Monash University receives from any commercialization of Phaxan.

This manuscript was handled by: Markus W. Hollmann, MD, PhD, DEAA.
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