Phaxan$_{CD}$™, a Captisol®-enabled water soluble preparation of alphaxalone for intravenous anesthesia and sedation: comparison of anesthetic properties with propofol and Althesin®

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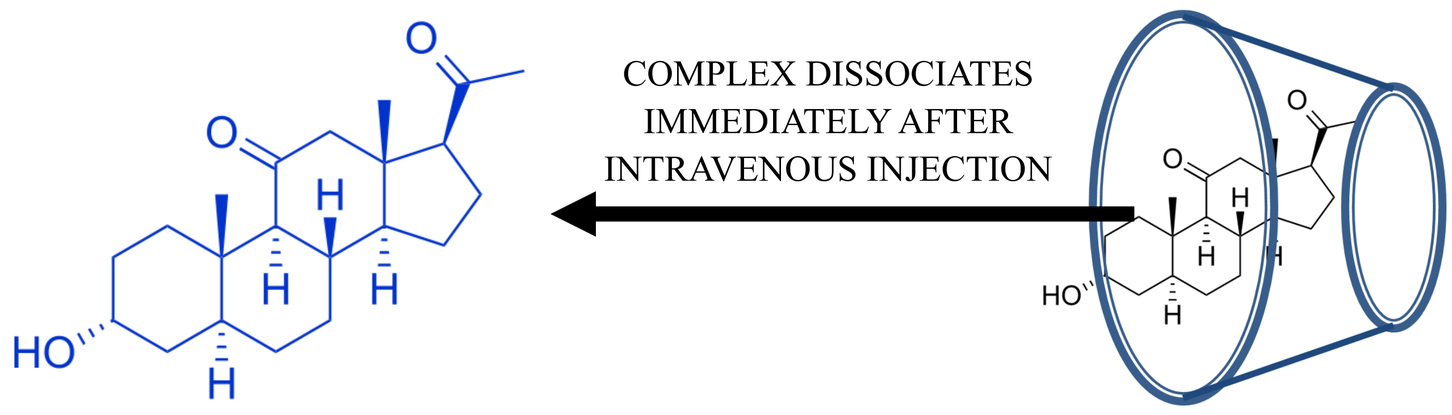
Introduction

Alphaxalone is a neuroactive steroid that causes anesthesia by positive modulation at $GABA_A$ receptors. It was the main ingredient of Althesin®, used widely for intravenous anesthesia from 1971 until 1984 when it was withdrawn from clinical practice because of hypersensitivity to Cremophor EL the emulsifying agent used in the formulation (1). Many attempts have been made to reformulate this compound for human use because of its favourable anesthetic and safety profile but hitherto those attempts have failed to prepare a suitable water based preparation. Captisol® is 7-sulfobutylether β-cyclodextrin; a molecule with a lipophilic cavity. that enables water insoluble drug dispersal in water for human use.

Objectives

1. dissolve alphaxalone in water using Captisol®
2. assess anesthesia and recovery using this preparation and
3. compare these properties with propofol and Althesin®



Materials and Methods

A solution of alphaxalone [10mg.ml^{-1} ;Phaxan$_{CD}$™ (PHAX)] was prepared by dissolving alphaxalone 300mg (0.9 mmoles) in saline 0.9% 30ml, using Captisol® (7-sulfobutyl ether beta cyclodextrin) 3889mg (1.8 mmoles); this produced a solution with a complexation ratio alphaxalone:Captisol® of 1:2. An "Althesin®-like" solution of alphaxalone in 20% CremophorEL (ALTH) was prepared as described in the literature (2). Male Wistar rats (wt 150-220g) were implanted with indwelling internal jugular vein intravenous catheters under halothane anaesthesia. Twenty four hours later each rat received an intravenous injection from a range of doses of either PHAX or ALTH (1.25, 2.5, 5, 10, & 15 mg.kg^{-1}), or propofol emulsion [10mg.ml^{-1} propofol; PROP] (1.25, 2.5, 3.75, 5, 10, 15, & 20mg.kg^{-1}); n = 10 rats at each dose. Three more groups of 10 rats each were given vehicle only instead of the anesthetic solutions [13% Captisol®; 20% CremophoEL; 10% lipid emulsion]. The following were assessed at regular time intervals after the intravenous injection:

- righting reflex: 1 normal; 2 slow; 3 some attempt; 4 none – this was a measure of onset and duration of unconsciousness
- tail pinch response: 1 normal; 2 weak; 3 just present; 4 none – this was a measure of onset and duration of surgical anaesthesia
- time the rat was able to walk on the rotarod (a rotating cylinder) measured in seconds: the maximal normal run time is 120 s in non sedated rats – this was a measure of time taken to attain full recovery from the sedating effects of the anaesthetic injections

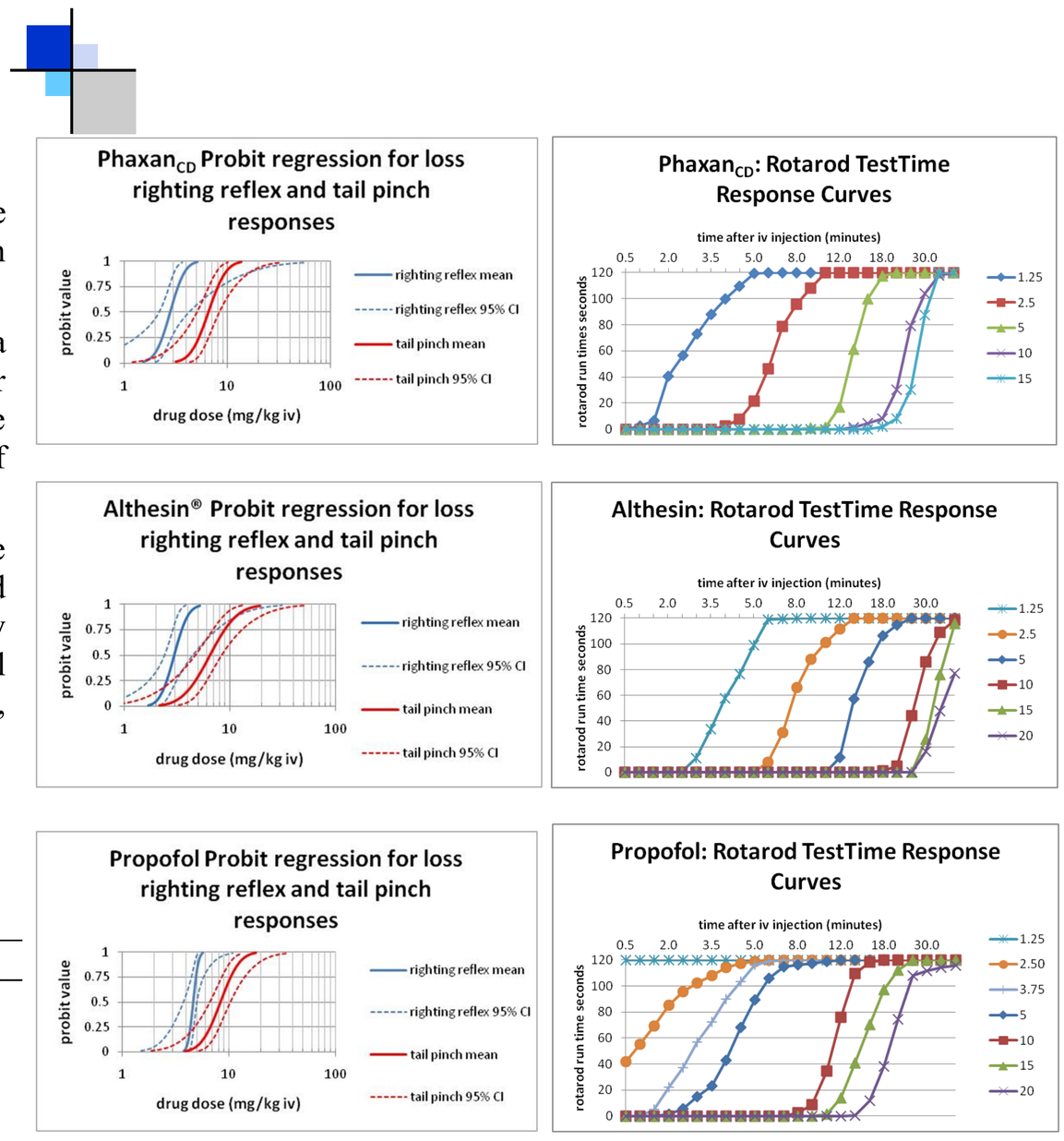
Results from groups of ten rats treated with the same anaesthetic and dose were combined for statistical purposes. Rats that attained a score of 4 for loss of righting reflex were deemed to have lost consciousness and those that scored a 4 for loss of tail pinch response were deemed to be surgically anaesthetised. The number of rats in each group of 10 similarly treated animals that scored 4 were subjected to probit regression analysis using SPSS Statistics 18 to produce graphs of probit value v log dose (probit plot). Those were used to calculate the estimated dose that caused anaesthesia in 50% and 95% of subjects (AD_{50} and AD_{95} respectively) for unconsciousness (righting reflex measurements) and surgical anaesthesia (tail pinch responses). The time of onset and recovery of rotarod walking ability was also plotted for each dose and treatment. This was used as a measurement of complete recovery.

Results

Alphaxalone (10mg.ml-1) dissolved readily in 13% Captisol®-saline solution. The resultant solution was colourless and completely clear. It did not cause stiction when used in plastic syringes.

Intravenous PHAX caused immediate dose-related sedation and anesthesia accompanied by no abnormal movements. The graphs show the probit plots for anesthetic end points for righting reflex and tail pinch responses. Also shown are graphs of the mean walking time on the rotarod for groups of 10 rats at each dose of the three anesthetic preparations.

The table below summarises the results taken from these graphical plots. It can be seen that Phaxan$_{CD}$ is equipotent with Althesin® in causing unconsciousness and surgical anaesthesia and both are more potent than propofol in this respect. Recovery from unconsciousness caused by Phaxan$_{CD}$ is just as fast as with propofol. Control experiments revealed that the vehicles given alone intravenously, 20% CremophorEL, 10% lipid emulsion and 13% Captisol had no sedating or anaesthetic effects.



	Althesin®	Phaxan$_{CD}$	propofol
dose causing all 10 rats to lose righting reflex mg.kg^{-1}	5	5	10
AD_{50} for loss of righting reflex mg.kg^{-1}	2.95	2.79	4.63
AD_{95} for loss of righting reflex mg.kg^{-1}	4.39	4.26	8.40
AD_{50} for loss of tail pinch response reflex mg.kg^{-1}	6.46	6.56	8.40
AD_{95} for loss of tail pinch response mg.kg^{-1}	14.09	8.56	14.46
duration (minutes) of loss of righting reflex at dose causing all 10 rats to lose righting reflex mean (SD)	3.6 (2.18)	1.9 (0.84)	2.5 (1.15)

Conclusions

Phaxan$_{CD}$™ causes anesthesia with fast onset, and offset timing equal with propofol and Althesin®, the former commercial human anesthetic formulation of alphaxalone.

Phaxan$_{CD}$™ is twice as potent as propofol.

Phaxan$_{CD}$™ is a clear water-soluble preparation using an FDA-approved excipient to achieve water solubility—Captisol®. This avoids the only impediment to alphaxalone being used clinically for human anesthesia in todays operating rooms, i.e., hypersensitivity reactions to CremophorEL. This advance will allow the reintroduction of this useful agent into human anesthetic practice.

Phaxan$_{CD}$™ is filterable with none of the infection, manufacturing and lipid toxicity issues associated with propofol lipid formulations.

Reference List

(1) Prys-Roberts C, Sear J. Steroid anaesthesia. Br J Anaesth 1980 Apr;52(4):363-5.

(2) Davis B, Pearce DR. An introduction to Althesin (CT 1341). Postgrad Med J 1972 Jun;48:Suppl-7.