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Formulation and evaluation of a propanidid hydroxypropyl-β-cyclodextrin solution for intravenous anaesthesia

Craig R. MacKenzie, J. Paul Fawcett *, David W. Boulton, Ian G. Tucker

School of Pharmacy, University of Otago, P.O. Box 913, Dunedin, New Zealand

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Abstract

Propanidid is a short acting anaesthetic with poor solubility in water. It was formerly marketed for clinical use as an intravenous (i.v.) anaesthetic in Cremophor EL® (Epontol®, propanidid 50 mg ml^{-1}) but this was withdrawn due to anaphylactoid reactions to the vehicle. This paper reports on an aqueous formulation using hydroxypropyl-β-cyclodextrin (HP-β-CD) as a solubiliser. Phase solubility analysis showed a linear increase in the solubility of propanidid to a maximum of about 80 mg ml^{-1} (15 times its solubility in water) in 50% w/v HP-β-CD. A 50 mg ml^{-1} solution in 42% w/v HP-β-CD was stable to autoclaving and on storage for at least 12 weeks at 42°C. The efficacy of the formulation as an i.v. induction agent was compared with that of a commercial propofol formulation (Diprivan®, propofol 10 mg ml^{-1}) in rats. Induction was rapid and independent of dose whereas sleep duration was dose dependent. Both induction time and sleep duration were significantly shorter for the propanidid formulation. The solution of propanidid in aqueous HP-β-CD is simple to prepare, stable and effective as a short acting i.v. anaesthetic. Its potential in clinical practice remains to be evaluated. © 1997 Elsevier Science B.V.

Keywords: Propanidid; Cyclodextrins; Propofol; Anaesthetics; Drug stability

1. Introduction

Propanidid (propyl 4-diethylcarbamoylmethoxy-3-methoxyphenylacetate, Fig. 1) was used as an ultra-short acting intravenous (i.v.) anaesthetic valued for its very fast onset of action and the rapid recovery from anaesthesia (Dundee and Clarke, 1964). The drug is a pale yellow, viscous oil with limited solubility in water (ca. 5 mg ml^{-1}) originally solubilised for clinical use (Epontol®, propanidid 50 mg ml^{-1}) by adding 20% of polyethoxylated castor oil (Cremophor EL®) (Conway and Ellis, 1970). This formulation

* Corresponding author. Tel.: + 64 3 4797290; fax: + 64 3 4797034; e-mail: paul.fawcett@stonebow.otago.ac.nz

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was subsequently withdrawn from use in many countries in the 1980s due to an association with anaphylactoid reactions (Christmas, 1984) shown to be due to the solubilising agent (Glen et al., 1979). With the growth in use of short-acting i.v. anaesthetics, investigation into reformulation of propanidid solution is warranted.

Complexation with an appropriate cyclodextrin (CD) can improve the aqueous solubility of many hydrophobic drugs (Albers and Müller, 1995). Hydroxypropyl-β-cyclodextrin (HP-β-CD) is particularly useful in this regard because of its relatively high solubility and low toxicity on i.v. administration (Brewster et al., 1990; Bekers et al., 1991). A number of studies have shown that HP-β-CD can dramatically increase the aqueous solubility of anaesthetic agents such as etomidate (Doenicke et al., 1994), propofol (Viernstein et al., 1993) and alfaxolone (Brewster et al., 1989) with little effect on their pharmacodynamic profiles. These investigations suggest that a CD complex of propanidid may be sufficiently soluble to be clinically useful.

The aim of this study was to determine the extent to which the solubility of propanidid in water could be increased using HP-β-CD and to evaluate an aqueous solution of propanidid as an i.v. anaesthetic agent. A propanidid 50 mg ml^{-1} formulation was prepared and its stability to sterilisation by autoclaving and on storage for 12 weeks at various temperatures was examined. The efficacy of the solution as an i.v. anaesthetic agent was compared with that of a commercial propofol formulation (Diprivan®, propofol 10 mg ml^{-1} in an aqueous emulsion) by determination of induction time and sleep duration in the rat.

2. Methods

2.1. Materials

Propanidid BP (1988) was a gift from B Braun Melsungen (Melsungen, Germany). HP-β-CD with an average degree of substitution of 3.29 was a gift from American Maize Products (Hammond, IN, USA). It was dried over phosphorous pentoxide for 48 h before being stored over silica gel. High performance liquid chromatography (HPLC) grade acetonitrile was purchased from Ajax (Clyde, Auburn, NSW, Australia). Distilled deionised water was produced by a reverse osmosis Milli-Q® Reagent Water system (Millipore®, Bedford, MA). Water for Injection BP was purchased from Delta West Pty (Bently, WA, Australia). Diprivan® was purchased from ICI (Auckland, New Zealand). Intralipid® 20% was purchased from Baxter Healthcare (Auckland, New Zealand). Male Sprague–Dawley rats were purchased from the Laboratory Animal Science Department, University of Otago.

2.2. Phase solubility analysis

The phase solubility study was performed according to the method of Higuchi and Connors (1965). Aliquots (40–1000 μl) of HP-β-CD solution (50% w/v) were added to constant amounts of propanidid (100 mg) and made up to final volumes of 1 ml with deionised water in plastic sample tubes ($n = 3$ for each concentration). The tubes were shaken in a water bath at 30 ± 0.5°C for 24 h before being centrifuged at $5700 \times g$ for 1 min. Aliquots of supernatant (200 μl) were diluted 1:1000 with mobile phase and analysed by HPLC. The solubility of propanidid increased to 79.9 mg ml^{-1} in the presence of 50% w/v HP-β-CD.

2.3. Formulation, sterilisation and stability

A 50 mg ml^{-1} formulation was prepared under clean conditions by adding 2 g propanidid to 40 ml of a 42% w/v HP-β-CD solution in Water for Injection BP. Aliquots (1 ml) were transferred into clean glass ampoules and sealed. The ampoules were then sterilised by autoclaving at 121°C for 15 min. A 42% w/v HP-β-CD solution and Water for Injection BP were also autoclaved as controls. The stability of the formulation and

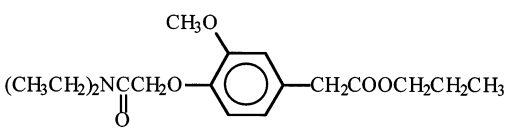

Fig. 1. Chemical structure of propanidid.

control solutions before and after autoclaving were examined by HPLC and pH measurement. Ampoules of the formulation for use in rats were stored in the refrigerator.

To assess the stability of the autoclaved formulation on long term storage, ampoules ($n = 18$) were placed in incubators at each of three temperatures, 4.0 ± 0.2, 25 ± 1.0 and 42.7 ± 0.2°C. Ampoules ($n = 3$) were removed from each storage condition initially and after 1, 2, 5, 8 and 12 weeks and frozen at -84 ± 1°C until analysis. Thawed samples were diluted 1:1000 with mobile phase and analysed by HPLC. Analysis of time zero samples stored at -84 ± 1°C showed no detectable decomposition.

2.4. HPLC assay of propanidid

Propanidid was analysed by a validated, stability indicating HPLC assay based on that of Nousiainen and Raatikainen (1984). The HPLC system was operated at ambient temperature and included a Hichrom ODS-2 (C_{18}) 25 cm × 4.6 mm stainless steel column (Hichrom, Reading, Berks, UK) and a UV detector at 280 nm. Using a mobile phase of 70% v/v acetonitrile and 30% v/v water at 1 ml min^{-1}, propanidid eluted at 3.9 min. The assay based on peak height was linear in the range 1–100 μg ml^{-1} and had intraday and interday variability of 1.5 and 5.4% respectively at 100 μg ml^{-1}.

2.5. Comparison of the propanidid HP-β-CD formulation with Diprivan® in rat

Approval for the study was obtained from the Committee on Ethics in the Care and Use of Laboratory Animals, University of Otago. Male Sprague–Dawley rats ($n = 30$, mean weight of 265 ± 19 g) were randomly assigned to receive either the propanidid 50 mg ml^{-1} formulation ($n = 12$), Diprivan® ($n = 12$) or control formulations ($n = 3$) of either 42% w/v HP-β-CD or Intralipid® 20%. The two drug administration groups were then randomly allocated into four sub-groups of three rats each. A 4×4 Latin Square was used to determine a random dose allocation of all doses to each of the sub-groups. The doses of propanidid used were 20, 34, 59 and 100 mg kg^{-1} (0.059–0.296 mmol kg^{-1}). The doses of propofol in Diprivan® were 5, 8.5, 15 and 25 mg kg^{-1} (0.028–0.140 mmol kg^{-1}). There was a 1 week washout period between administration of each dose. Rats were supplied with food and drink ad libitum.

Each animal was weighed and administered the appropriate dose via the lateral saphenous vein at a rate of 0.5 ml min^{-1}. Induction time was taken from the time of commencement of the injection to the time the tail went limp. After drug administration, animals were placed on a heated pad. Analgesia was determined immediately following the end of drug administration by the same investigator using the pedal-withdrawal test where a hind toe of the animal was pinched with a pair of forceps. The response to this stimuli was graded from one (strong response, no analgesia) through to four (no response, strong analgesia). Duration of sleep was determined as the time for re-establishment of the righting reflex.

2.6. Statistical analysis

Data are given as mean ± standard deviation (S.D.). Two-way repeated measures analysis of variance (Minitab v.10.2, Minitab, State College, PA) was used to determine the significance of time and temperature on the chemical stability of propanidid in the formulation on long term storage. Repeated measures multivariate analysis of variance (SPSS v.4.0 for DEC station, SPSS, Chicago, IL) was used to assess the significance of formulation type, dose and period of administration on the induction time and duration of sleep produced by each formulation. The level of significance was set at $p = 0.05$.

3. Results

The solubility of propanidid increased as a function of HP-β-CD concentration. Linear regression of the phase solubility profile (Fig. 2) showed a linear dependence on HP-β-CD concentration ($r^2 = 0.990$, $p < 0.05$). The slope was 0.60 and the intercept 21 mM. Propanidid solubility

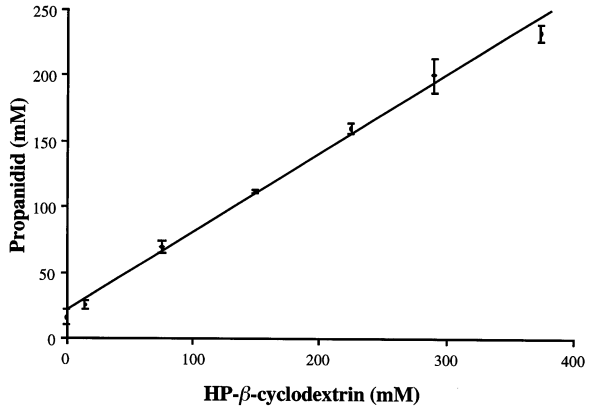

Fig. 2. Phase-solubility profile of propanidid as a function of HP-β-CD concentration at 30°C. (Each point represents mean $\pm$ S.D for $n = 3$)

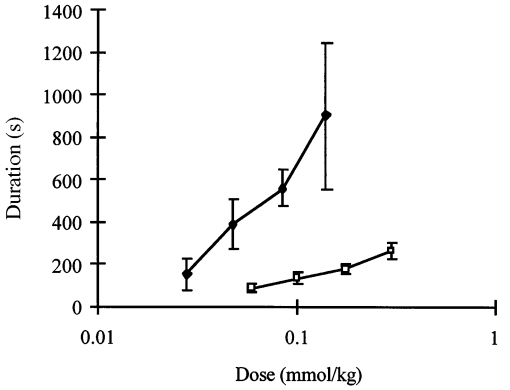

Fig. 3. The duration of sleep as a function of dose of propanidid in the propanidid HP-β-CD formulation (*) and of propofol in Diprivan® (◆). (Data are mean $\pm$ S.D., $n = 12$).

increased from 16.1 (5.4 mg ml^{-1}) to 233 mM (79.9 mg ml^{-1}) at a HP-β-CD concentration of 374 mM (50% w/v). The association constant assuming a 1:1 complex was calculated to be 70 M^{-1}.

Autoclaving of the 50 mg ml^{-1} propanidid formulation for 15 min caused a significant decrease in pH from 7.02 ± 0.04 to 5.42 ± 0.02 but there was no detectable decrease in the concentration of propanidid as shown by HPLC. The pH of the 42% w/v HP-β-CD solution and Water for Injection controls showed no significant change after autoclaving. Autoclaving the propanidid formulation for longer periods did not lead to further change in pH or to any detectable decomposition of propanidid. No significant change in pH or decomposition of propanidid was observed in formulations stored for 12 weeks.

With respect to the pharmacodynamic study, there was an effect of period for both induction time and sleep duration for both propanidid and propofol formulations presumably due to the effects of increasing age and mass of the rats over the 4 week study. The induction time was independent of dose for both formulations. The induction time for the propanidid formulation was 11.9 ± 2.1 s which was significantly faster than the 18.5 ± 3.9 s observed for Diprivan®.

The effect of dose on duration of sleep is shown in Fig. 3. There was a significant increase in sleep duration with increasing dose for both formulations. The sleep duration produced by propofol in Diprivan® was significantly longer than for an equimolar dose of propanidid ($p < 0.001$). The sleep duration produced by the highest dose of propanidid was 267 ± 39.5 s compared with that produced by the highest dose of propofol in Diprivan® of 904 ± 343.5 s. In terms of analgesia, dose-response relationships were observed in the pedal-withdrawal test for both formulations with propofol in Diprivan® appearing to be somewhat more potent (Table 1).

Table 1
Analgesic response immediately following the end of drug administration for the propanidid HP-β-CD formulation and Diprivan® as measured by pedal-withdrawal in rats

Dose (mmol kg^{-1})	Pain response[a]
Propanidid	
0.059	1.3 ± 0.5
0.101	2.4 ± 0.8
0.175	3.3 ± 0.7
0.296	3.8 ± 0.8
Propofol in Diprivan®	
0.028	1.6 ± 0.8
0.048	3.5 ± 0.5
0.084	3.7 ± 0.7
0.140	3.9 ± 0.4

Data are mean $\pm$ S.D., $n = 12$.
[a] Graded from 1 (no analgesia) through to 4 (strong analgesia).

4. Discussion

HP-β-CD has been previously shown to increase the solubility of poorly soluble compounds (Albers and Müller, 1995). In this study, the slope of less than one for the phase solubility profile suggests a 1:1 complex is formed between propanidid and HP-β-CD. The association constant of the complex of 70 M^{-1} implies a very weak association between the two molecules. Whilst this could be an advantage in terms of dissociation in vivo, it appears HP-β-CD complexes of anaesthetic drugs with very high association constants retain the anaesthetic efficacy of the uncomplexed drug (Brewster et al., 1990; 1995).

The decrease in pH on autoclaving the propanidid HP-β-CD formulation for 15 min may be due to ester hydrolysis of propanidid to the aromatic carboxylic acid. Assuming a pK_a of 4.2 for this acid, only a small amount of decomposition of propanidid ($< 0.002\%$) is necessary to account for the observed drop in pH and this was not detectable by HPLC. The formulation was shown to be very stable on long term storage even at temperatures of up to 42°C. This suggests the formulation can be sterilised for use and stored for reasonable periods of time without significant decomposition.

The propanidid formulation was shown to have a faster induction time than Diprivan®. Studies in humans have shown that induction with propanidid is extremely rapid (equivalent to one arm-brain time) (Conway and Ellis, 1970). The absence of a dose-response relationship for induction time in our study is probably a consequence of the slow rate of drug administration. Consistent with earlier studies, the duration of anaesthesia increased linearly with increasing log dose for both formulations and the duration of action of the propanidid formulation was significantly shorter than that produced by an equimolar dose of propofol in Diprivan® (Deschodt et al., 1988).

HP-β-CD appears to be less toxic than most other cyclodextrins but there is still concern regarding renal toxicity arising from its precipitation in the renal tubules (Brewster et al., 1990). Clinical experience with propanidid suggests that an infusion of 5–10 mg kg^{-1} is a commonly used dose as an induction agent or as the sole anaesthetic agent in short procedures (Howells et al., 1964). On this basis, the administration of our formulation would provide a dose in the order of 84 mg kg^{-1} of HP-β-CD. Acute dosing in monkeys of up to 10 g kg^{-1} of HP-β-CD produced no demonstrable toxic manifestations. Other formulations of i.v. anaesthetic agents solubilised with HP-β-CD involve similar concentrations of cyclodextrin but their clinical safety remains to be demonstrated.

In summary this study has demonstrated that a propanidid 50 mg ml^{-1} aqueous solution in 42% w/v HP-β-CD is an effective i.v. anaesthetic formulation with a shorter induction time and duration of action than an equimolar dose of propofol in Diprivan®. The formulation is simple to prepare and stable to autoclaving and on long term storage. It may provide some impetus for the reintroduction to clinical practice of a useful short acting i.v. anaesthetic.

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