J. Pharm. Pharmacol. 1996, 48: 529–531
Received August 25, 1995
Accepted January 19, 1996

Selective Cyclodextrin Inhibition of Alfaxolone-induced Ataxia

LEE KOETZNER, RAINE L. RIGGS, JOHN MASTROPAOLO, STEPHEN I. DEUTSCH*

*Behavioral Pharmacology Laboratory/Psychiatry Service 116A, VA Medical Center, 50 Irving Street NW, Washington, DC 20422 USA and *Department of Psychiatry, Georgetown University School of Medicine, 3800 Reservoir Road NW, Washington, DC 20007, USA*

Abstract

The effect of the use of a number of popular solubility treatments was examined on alfaxolone- and diazepam-induced ataxia.

The effects of diazepam were not significantly altered by solution in cyclodextrin, Alkamuls EL-620 or a mixture of propylene glycol and ethanol. The effects of alfaxolone were not altered by solution in Alkamuls EL-620, but were significantly lessened by solution in cyclodextrin. In a dose-response experiment, the ED50 of alfaxolone increased from 15·3 mg kg^{-1} (in Alkamuls EL-620) to 25·6 mg kg^{-1} (in hydroxypropyl-β-cyclodextrin).

The results suggest that although cyclodextrins are popular and effective solubilizers, their use must be considered carefully in the context of the experiments in which they are to be used.

The introduction of cyclodextrin solutions to behavioural pharmacology has offered a chance to solve a long-standing difficulty in the field: the low solubility of nonpolar drug molecules such as steroids. As these drugs are both therapeutically useful and of great interest to researchers, many steroid-cyclodextrin complexes have been used in research (Szejtli 1988). The parent cyclodextrin molecules (such as the heptamer β-cyclodextrin) dramatically increase the amount of steroid that could be solvated in a fixed volume of fluid (Pitha et al 1986). Further development led to substituted molecules which are easier to handle and are themselves more water-soluble; the best combination of these properties appears to be hydroxypropyl-β-cyclodextrin (Pitha & Pitha 1985). The hydroxypropyl derivative is readily water-soluble in large quantities (up to 75 % by weight; Pitha et al 1986) and has the added benefit of not showing toxic effects in mice after chronic administration (Pitha & Pitha 1985).

While there is no doubt that cyclodextrin solutions have been widely used both in medicine and in research (Szejtli 1988), there have been slight differences in results obtained by laboratories investigating steroids in cyclodextrin solutions and laboratories investigating steroids in other types of solution. A pertinent example is the use of drug discrimination procedures in rats to compare neuroactive steroids with benzodiazepines. One laboratory has found that the neurosteroid allotetrahydrodeoxycorticosterone, dissolved in dimethylsulphoxide, substitutes for the benzodiazepine midazolam (Deutsch & Mastropaolo 1993); another laboratory has found that the same neurosteroid, dissolved in cyclodextrin, does not substitute for two other benzodiazepines, lorazepam and diazepam (Ator et al 1993).

These differences in results are ambiguous. While they can be taken as evidence that cyclodextrin solutions are somehow different from other solutions of the same drug, they can also be interpreted as a result of many minor procedural differences between laboratories. In order to resolve this ambiguity, a series of experiments was designed to compare the effects of drugs in different solutions in the same procedure. Doses of 3·2 mg kg^{-1} diazepam and 25 mg kg^{-1} alfaxolone, which pilot tests had shown to be approximately the ED50 for each drug, were administered to groups of mice which were then tested for ataxia in the rotating rod assay. Diazepam was tested in solutions of hydroxypropyl-β-cyclodextrin, Alkamuls EL-620 and a combination of propylene glycol and ethanol; alfaxolone was tested in solutions of cyclodextrin, Alkamuls and dimethylsulphoxide. Further comparisons of vehicle effects were made by testing a series of doses of alfaxolone in solutions of cyclodextrin and Alkamuls.

Correspondence: S. I. Deutsch, Psychiatry Service 116A, VA Medical Center, 50 Irving Street NW, Washington DC 20422, USA.

Materials and Methods

Subjects

Outbred male mice (NIH:Swiss, Charles River, Frederick, MD) were used in these experiments. Animals were maintained in groups of five in hanging wire cages with food and water freely available, and were approximately ten to fourteen weeks of age (approximately 20–30 g in weight) at the time of the experiment. Groups of fourteen to seventeen animals (after training and selection) were used for each condition.

Procedure

The experiment began with a 5 min training segment. During this period, mice were placed on a rod rotating at a constant 12 rev min^{-1} (Ugo Basile 7600, Varese, Italy). If the animal fell from the rod, it was returned to the rod; if the animal fell four times in five minutes, it was excluded from the experiment. After training, each animal was injected with one of the treatments under study and returned to the transport cage. When ten minutes had elapsed, the animal was again placed on the rotating rod. If the animal then fell before ten seconds had passed, it was returned to the rod and the counter restarted; this was only done once, and the longer of the two times was recorded. If the animal did not fall after five minutes, it was removed from the rod and a value of 300 s was recorded.

Table 1: Effect of several solvents on diazepam- and alfaxolone-induced ataxia as measured by a rotating rod assay. A 3·2 mg kg^{-1} dose of diazepam was dissolved in 450 mg mL^{-1} hydroxypropyl-β-cyclodextrin, 10% v/v Alkamuls EL-620 and 10% propylene glycol with 5 % ethanol (both v/v). A 25 mg kg^{-1} dose of alfaxolone was dissolved in the same concentrations of cyclodextrin and Alkamuls and also in 50% v/v dimethylsulphoxide. The table shows the mean time that the mice in each condition were able to stay on the rotating rod and the s.e.m.

	Time on the rotorod		
Solvent	Vehicle	Diazepam	Alfaxolone
Cyclodextrin	289·3 (10·72)	145·0 (32·60)	148·2 (29·42)
Alkamuls	282·4 (15·16)	105·1 (29·42)	4·2 (0·81)
PEG/EtOH	268·7 (16·26)	106·8 (21.04)	
Dimethylsulphoxide	117·6 (32·79)		2·3 (0·63)

Drugs and solvents

Diazepam free base (Hoffmann-La Roche, Nutley, NJ) was dissolved in 10% v/v Alkamuls EL-620 (formerly Emulphor EL620; Rhone-Poulenc, Cranbury, NJ), 450 mg mL^{-1} hydroxypropyl-β-cyclodextrin (Research Biochemicals, Natick, MA) and a combination of 10% v/v propylene glycol (Sigma, St Louis, MO) and 5% v/v ethanol (Pharmco, Brookfield, CT). Alfaxolone (Glaxo, Middlesex, UK) was dissolved in 10% v:v Alkamuls, 450 mg mL^{-1} cyclodextrin and 50 % v:v dimethylsulphoxide (Sigma, St Louis, MO). All of these substances were dissolved in double-distilled, deionized water and injected intraperitoneally.

Data analysis

Assessment of drug and solvent effects was conducted with two-way analyses of variance; Scheffé tests were used for post-hoc significance testing. Dose-response data were also analysed by fitting four-parameter logistic equations to the data; these logistic models accounted for a minimum of 95·7% of the variability in the data. The α criterion for hypothesis testing was set at $P < 0.05$.

Results

Diazepam vehicle comparisons

Variation of the vehicle used to dissolve diazepam did not significantly affect performance ($F(2,88) = 0.194$, $P > 0.82$). Administration of a 3·2 mg kg^{-1} dose of diazepam decreased the amount of time spent on the rod ($F(1,88) = 89.928$, $P < 0.001$) but did not interact significantly with the vehicle ($F(2,88) = 0.075$, $P > 0.92$). Inspection of Table 1 reveals a mean time of 145 s on the rod for animals receiving diazepam in cyclodextrin, but this time is not significantly greater than the times for animals receiving diazepam in Alkamuls (105·1 s, $P > 0.99$) or the combination of propylene glycol and ethanol (106·8 s, $P > 0.99$).

Alfaxolone vehicle comparisons

Administration of a dose of 25 mg kg^{-1} of alfaxolone produced a marked decrease in the amount of time spent on the rod ($F(1,84) = 117.656$, $P < 0.001$). However, the vehicle used to dissolve alfaxolone also produced a significant effect ($F(2,84) = 30.576$, $P < 0.001$). The interaction between the two ($F(2,84) = 9.571$, $P < 0.001$) is therefore somewhat ambiguous. As can be seen in Table 1, the dimethylsulphoxide vehicle had a disruptive effect relative to the cyclodextrin vehicle ($P < 0.001$) and to the Alkamuls vehicle ($P < 0.001$). However, animals treated with the Alkamuls vehicle did not display any more or less disruption than animals treated with the cyclodextrin vehicle (Alkamuls, 282·4 s; cyclodextrin, 289·3 s; $P > 0.999$). There were also significant differences among groups of animals treated with different solutions of alfaxolone. Animals treated with alfaxolone in cyclodextrin showed markedly less behavioural disruption (mean time, 148·2 s) than animals treated with the same dose of alfaxolone in Alkamuls (4·2 s, $P < 0.001$) or dimethylsulphoxide (2·3 s, $P < 0.001$).

Alfaxolone dose-response comparison

Increasing doses of alfaxolone resulted in increasing degrees of ataxia ($F(3,116) = 26.104$, $P < 0.001$; see also Fig. 1). However, when the drug was administered in solutions of both cyclodextrin and Alkamuls, the cyclodextrin solution was less effective ($F(1,116) = 13.861$, $P < 0.001$). In addition, there was a significant interaction between vehicle and drug ($F(3,116) = 3.170$; $P < 0.03$). Groups treated with lower doses of alfaxolone did not show statistically significant differences (vehicle: $P > 0.999$; 10 mg kg^{-1}: $P > 0.999$; 20 mg kg^{-1}: $P > 0.43$), although groups treated with 25 mg kg^{-1} of alfaxolone did

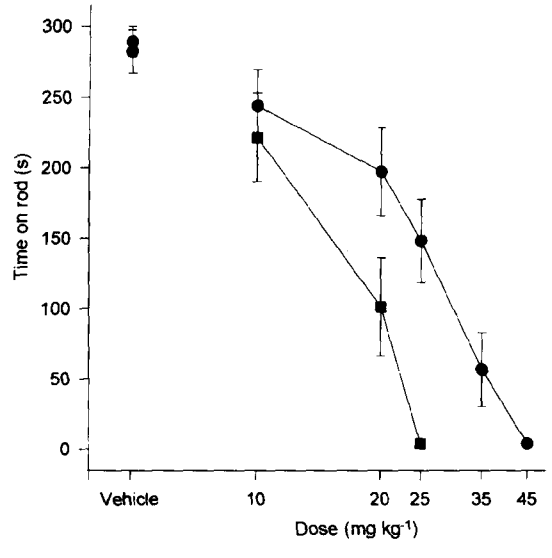

FIG. 1. Dose-response curves for alfaxolone dissolved in 450 mg mL^{-1} hydroxypropyl-β-cyclodextrin and 10% v:v Alkamuls EL-620. (●) Cyclodextrin solution; (■) Alkamuls solution.

($P < 0.03$). These differences were also reflected in the parameters returned by the logistic regression. Alfaxolone dissolved in cyclodextrin displayed an ED50 of 25·6 mg kg^{-1}; dissolved in Alkamuls, the ED50 was 15·3 mg kg^{-1}. The shallower slope of the dose-response curve for alfaxolone dissolved in cyclodextrin was reflected in a larger slope parameter (4·47 compared with 3·75 for alfaxolone dissolved in Alkamuls).

Discussion

The most important result of this experiment is that cyclodextrin can reduce the effect of a drug which is dissolved in it. The solution of 25 mg kg^{-1} of alfaxolone in cyclodextrin was markedly less effective in producing disruption than solutions in either Alkamuls or dimethylsulphoxide. It could be argued that dimethylsulphoxide merely added its own disruptive effect to that of the steroid; however, the same cannot be said for Alkamuls. In addition, the use of a cyclodextrin vehicle markedly changed the dose-response curve for alfaxolone, increasing its ED50 relative to the Alkamuls vehicle by two thirds, from 15·3 to 25·6 mg kg^{-1}. At the same time, the slope of the dose-response curve changed from a steep descent to a much slower drop; this indicates that a second binding site might be present (Berenbaum 1989). The nonpolar interior of the cyclodextrin molecule could easily be the second site.

Interestingly, this diminution of drug effect is selective. Diazepam dissolved in cyclodextrin seemed slightly less effective than diazepam dissolved in either Alkamuls or propylene glycol with ethanol; however, this was not statistically significant. This difference between diazepam and alfaxolone cannot be explained by the present data, but a hypothesis can be formulated. Drugs which bind to the cyclodextrin molecule with particularly high affinity are not as readily available to the body as their solubility in a cyclodextrin solution would indicate (Szejtli 1988). Alfaxolone – and possibly other steroids – may simply bind to cyclodextrin molecules with far higher affinity than diazepam. Diazepam is a smaller molecule, and it may be limited to a looser fit than alfaxolone. Alternately, alfaxolone may simply present more nonpolar atoms to the interior of the cyclodextrin. The determinants of drug binding to cyclodextrin are partly understood (Szejtli 1988), but their contribution to drug effectiveness is not.

This line of reasoning leads to the first possibility for improving bioavailability of drugs in cyclodextrin solutions: the use of the octameric hydroxypropyl-γ-cyclodextrin. As the γ form is one monomer larger than the β form, it has a larger interior; this might be more appropriate for a molecule the size and shape of a steroid. Alternately, lower concentrations of cyclodextrin may result in greater drug effects. In the present experiment, the molar ratios of drug:cyclodextrin were 1:41.1 for alfaxolone (2·5 mg mL^{-1} at 25 mg kg^{-1}) and 1:273.9 for diazepam (0·32 mg mL^{-1} at 3·2 mg kg^{-1}). It is possible that complex formation is so much more energetically favourable that when a drug molecule dissociates from a molecule of cyclodextrin, the re-formation of a complex (with the original molecule or with one of the excess, uncomplexed cyclodextrins also in solution) is the favoured pathway. A far less concentrated solution of cyclodextrin may reduce this problem.

These data provide a framework for understanding some of the differences in results from different laboratories using similar procedures to investigate the same steroids. The data also suggest that for concentrations of steroid where Alkamuls is sufficient to dissolve the drug, it is the preferred vehicle; for higher concentrations, enhancements to the solubilizing procedure may be possible. However, as these issues are addressed, further questions arise. The relative biological effect of drugs can only be related to their affinity for cyclodextrins by further study. Likewise, further study is required to delineate the effects of cyclodextrin size and concentration on drug effect.

Acknowledgments

Diazepam was generously provided by Dr Peter Sorter of Hoffmann-La Roche; alfaxolone was a gift of Dr B. M. Bain of Glaxo. Alkamuls EL-620 (formerly Emulphor) was provided as a sample by Rhone-Poulenc. This work was supported by grants from the Department of Veterans Affairs to S. I. Deutsch.

References

Ator, N. A., Grant, K. A., Purdy, R. H., Paul, S. M., Griffiths, R. R. (1993) Drug discrimination analysis of endogenous neuroactive steroids in rats. Eur. J. Pharmacol. 241:237–243

Berenbaum, M. C. (1989) What is synergy? Pharmacol. Rev. 41: 93–141

Deutsch, S. I., Mastropaolo, J. (1993) Discriminative stimulus properties of midazolam are shared by a GABA-receptor positive steroid. Pharmacol. Biochem. Behav. 46: 963–965

Pitha, J., Pitha, J. (1985) Amorphous water-soluble derivatives of cyclodextrins: nontoxic dissolution enhancing excipients. J. Pharm. Sci. 74: 987–990

Pitha, J., Milecki, J., Fales, H., Pannell, L., Uekama, K. (1986) Hydroxypropyl-β-cyclodextrin: preparation and characterization; effects on solubility of drugs. Int. J. Pharm. 29: 73–82

Szejtli, J. (1988) Cyclodextrin Technology. Kluwer Academic Publishers, The Netherlands