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Stabilisation of hydrotropic temazepam parenteral formulations by lyophilisation

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Summary

The solubilisation of temazepam by hydrotropic complexation has been investigated. Sodium salicyate and nicotinamide were used as hydrotropes. The former allowed appreciable solubilisation of temazepam but the effect was less marked in the case of diazepam. Increased solubilisation with temazepam was attributed to an increase in hydrogen bonding between drug and hydrotrope. Solutions of temazepam solubilised with sodium salicylate developed an unacceptable yellow colour on storage. This problem was overcome by lyophilisation. Lyophilised injections were readily reconstituted and initial assessment in rabbits indicated a satisfactory pharmacological response. Lyophilisation resulted in a preparation with excellent storage characteristics.

1. Introduction

Benzodiazepines are one of the most widely used groups of drugs in medical practice. Certain of these drugs are of particular value as intravenous premedicants for minor surgical and investigative procedures such as gastroscopy. The intravenous route provides the most rapid onset of sedative action. Ideally, a drug possessing an intermediate duration of action with no risk of active metabolite accumulation is required. Relatively long-acting intravenous diazepam is currently widely used as a surgical premedicant. However, patients often exhibit hang-over symptoms following diazepam treatment. In contrast, however, the short-acting midazolam may require repeated intravenous administration to maintain adequate sedation of the patient. Temazepam (3-hydroxy-diazepam) is a benzodiazepine tranquiliser which has been shown to possess the appropriate pharmacokinetic properties for such procedures, with a rapid onset of action and a fairly short elimination half-life resulting in an intermediate duration of action (Pickup et al., 1984).

Temazepam has both poor aqueous solubility and stability. Although it has been shown that a stable parental temazepam formulation may be prepared using a 80/20 propylene glycol–water mixture (McCafferty et al., 1986) such preparations have high viscosity and are therefore difficult to inject. A further problem may be the risk of precipitation of drug microcrystals on transition

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from the water-miscible organic solvent phase (injection) to the aqueous phase (blood) with consequent adverse venous sequelae. Clearly, an aqueous temazepam preparation may be advantageous. The hydrotropic solubilisation approach (Neuberg, 1916; Windsor, 1950) has been applied to several benzodiazepines (Badwan et al., 1980). The present study investigates this approach with specific respect to temazepam using lyophilisation to overcome problems of inadequate aqueous stability.

Materials, Methods and Results

Chemicals

Temazepam was a gift of Farmitalia Carlo Erba Ltd. All other chemicals were of pharmacopoeial or analytical reagent grade.

Phase diagrams for temazepam complexation

Sufficient temazepam was weighed accurately into each of several flasks to give a final possible solution concentration of 25 $mg \cdot ml^{-1}$. The appropriate complexing ligand was added to give final ligand concentrations ranging from 5 to 35% m/V. Water for injection was added to produce a final volume of 25.0 ml. Subsequently, each flask was sealed and shaken for 3 h at 22°C. Solutions were then filtered through a 0.45 μm filter (Millipore U.K.) and the solution phase concentration of temazepam determined by HPLC as previously described (McCafferty et al., 1986). Temazepam was solubilised with sodium salicylate and then with nicotimanide as the ligand (Fig. 1). For comparison, the relative efficacy of these ligands at solubilising diazepam is shown in Fig. 2.

Preparation of lyophilised temazepam for parenteral use

Initial aqueous solutions were prepared by dissolving together appropriate amounts of temazepam, sodium salicylate and lactose. The preferred formulation contained temazepam (20 $mg \cdot ml^{-1}$), sodium salicylate (400 $mg \cdot ml^{-1}$) and lactose (100 $mg \cdot ml^{-1}$) in Water-for-Injection (q.s.). This solution was stable for at least 24 h at room temperature and 1 week when refrigerated. Prior to lyophilisation the initial solution was diluted 1 part in 2 with Water-for-Injection, filtered aseptically (0.22 μm, Millipore, U.K.) and 4 ml filled aseptically into sterile borosilicate glass freeze-drying vials with butyl rubber caps.

Lyophilisation was carried out using a modified Edwards EF1 shelf-dryer (Edwards High Vacuum Ltd.) and primary drying only. Vials were frozen for 30 min at −30°C. Sublimation then took place at 0.13 mbar and a condenser temperature of −50°C. Drying continued for 16 h (overnight) and vials were subsequently sealed under vacuum.

Reconstitution of lyophilised preparations

Lyophilised preparations were reconstituted with Water-for-Injection (2 ml). Dissolution of the lyophilised plug occurred easily within 15 s on gentle shaking of the vial. Initially, a variety of formulations were lyophilised and examined for ease of reconstitution and clarity. Results are presented in Table 1.

Dilution profiles of reconstituted injections

A series of dilutions with various diluents were prepared following initial reconstitution of a series of lyophilised temazepam formulations with

TABLE 1

RECONSTITUTION OF LYOPHILISED TEMAZEPAM FORMULATIONS

Temazepam (mg/ml)	Sodium salicylate (mg/ml)	PVP (mg/ml)	Lactose (mg/ml)	product clarity
10	300	–	–	slight opalescence
7.5	300	–	–	slight opalescence
5	250	–	–	slight opalescence
3.33	200	–	–	opalescence
2.5	200	–	–	opalescence
5	250	50	–	slight opalescence
5	250	–	10	opalescence
5	250	–	25	slight opalescence
5	250	–	50	clear
5	200	–	50	clear

TABLE 2

DILUTION PROFILES FOR LYOPHILISED TEMAZEPAM FORMULATIONS

Solution (mg/ml)	Diluent	Factor	Temazepam solubility
300S/100L/20T	W	1 : 2	ppt. 30 min
350S/100L/20T	W	1 : 2	ppt. 30 min
400S/100L/20T	W	1 : 2	remains in solution
400S/100L/20T	W	1 : 3	slight opalescence
400S/100L/20T	SAL	1 : 3	slight opalescence
450S/100L/20T	W	1 : 3	ppt. 15 min
450S/100L/20T	SAL	1 : 3 and 1 : 4	ppt. 45 min
500S/100L/40T	W	1 : 2	remains in solution
500S/100L/40T	W	1 : 3	ppt. 10 min
400S/100L/20T	5%PVP	1 : 5	ppt. instantly
	5%PVP/10%PG	1 : 5	ppt. instantly
	10%PVP/10%PG	1 : 5	ppt. < 5min
400S/100L/20T	10%PG	1 : 5	ppt. instantly
	20%PG	1 : 5	ppt. < 60 min
	30%PG	1 : 5	ppt. < 60 min
	40%PG	1 : 5	remains in solution

S = sod. salicylate; L = lactose; T = temazepam; W = water; SAL = saline; PVP = polyvinylpyrrolidone; PG = propylene glycol; PPT = precipitates.

Water-for-Injection. These were monitored visually for precipitation during storage at ambient temperature (Table 2).

Stability monitoring

Vials containing the preferred lyophilised formulation were stored at 4, 25 and 40 °C. They were reconstituted and assayed for intact temazepam at monthly intervals by the method previously described. No detectable loss of temazepam was found to have occurred in any of the stored samples over a 6 month period.

Assessment of lyophilised injections in rabbits

The preferred lyophilised temazepam formulation (temazepam 20 $mg \cdot ml^{-1}$) was assessed in rabbits for initial signs of efficacy and toxicity. Rabbits were New Zealand whites weighing approximately 4 kg. Intravenous injection was made via the ear vein. Following reconstitution 1 ml of the preparation was injected into each of 4 rabbits. There were some signs of slight irritation on injection. However, the rabbits were well sedated rapidly. They exhibited a diminished pain reflex and regular eye movement. Signs of recovery were apparent some 30 min after injection. On follow-up after 24 h all the rabbits were fully recovered and showed no apparent adverse reactions. Further examination over the next 3 months indicated no apparent damage at the injection site.

Discussion

The concept of hydrotropy due to Neuberg (1916) was originally confined to anionic organic salts which, when present in high concentrations, increased the aqueous solubility of poorly soluble solutes. More recently Saleh and El-Khordagui (1985) have extended the definition of hydrotropes to include soluble cationic agents with planar structures capable of stacking in solution. Hydrotropic solubilisation of some benzodiazepines, excluding temazepam, showed promising increases in aqueous solubility of the drugs (Badwan et al., 1980), suggesting the possible application of this technique to the formulation of aqueous parenteral temazepam.

Since the hydrotropic solubilisation of temazepam has not previously been studied phase dia-

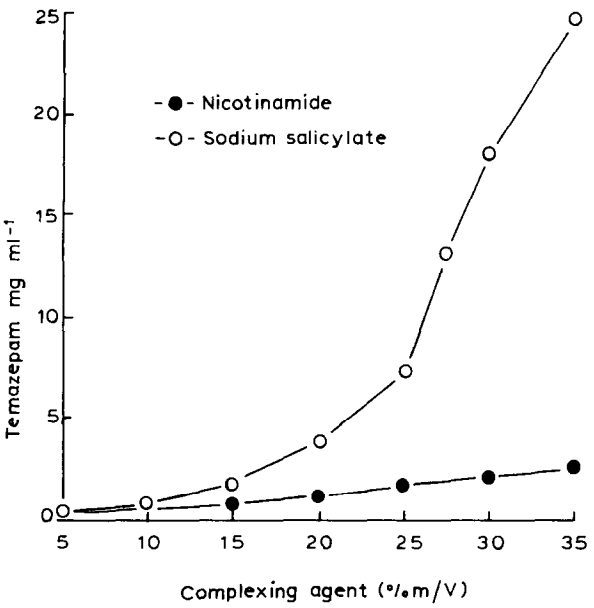

Fig. 1. Phase diagrams for the complexation of temazepam with sodium salicylate and nicotinamide.

grams of temazepam complexation with sodium salicylate were prepared (Figs. 1 and 2). Sodium salicylate has previously been identified as a suitable ligand for benzodiazepines (Badwan et al., 1980) and benzoates in general have been demonstrated to have hydrotropic properties (Saleh and El-Khordagui, 1985). A diverse range of compounds may act as hydrotropes (Attwood and Florence, 1983). Sodium salicylate has been shown to increase the aqueous solubility of many drugs including riboflavine (Attwood and Florence, 1983). Nicotinamide, structurally quite different, also has a marked effect on riboflavine solubility (Frost, 1947). Thus, in the present study nicotinamide was also investigated as a possible ligand. Both hydrotropes were also studied in terms of their comparative solubilising power for diazepam. Results indicated that nicotinamide was a poor solubilising agent for both diazepam and temazepam. However, there was a rapid rise in the amount of both diazepam and temazepam solubilised when sodium salicylate was present at a final solution concentration above 20% m/V. Phase diagrams for solubilisation of temazepam (Fig. 1) and diazepam (Fig. 2) show a typical positive deviation from linearity consistent with hydrotropic solubilisation (Elworthy et al., 1968). This solubilisation pattern probably results from the contribution of higher order complexes containing more than one ligand molecule (Higuchi and Connors, 1965). The contribution of these higher order complexes to drug solubility increases as the ligand concentration increases. Higher ligand concentrations may therefore be expected to produce a marked increase in drug solubilisation. Thus, sodium salicylate (30% m/V) was observed to solubilise approximately 18 $mg \cdot ml^{-1}$ of temazepam as compared with only 8 $mg \cdot ml^{-1}$ of diazepam. This is particularly interesting since temazepam is intrinsically less soluble in water than diazepam, probably due to intramolecular hydrogen bonding mediated via the 3-hydroxyl group. This group, however, also offers the possibility of additional hydrogen bonding between temazepam and the hydrotrope. In the case of nicotinamide the -NH group would be less efficient at forming such bonds and hence its poor solubilising effect on the benzodiazepines. The additional hydrogen bonding possible between temazepam and the salicylate may account for the increased solubility of the drug compared to diazepam. This effect would be additional to the

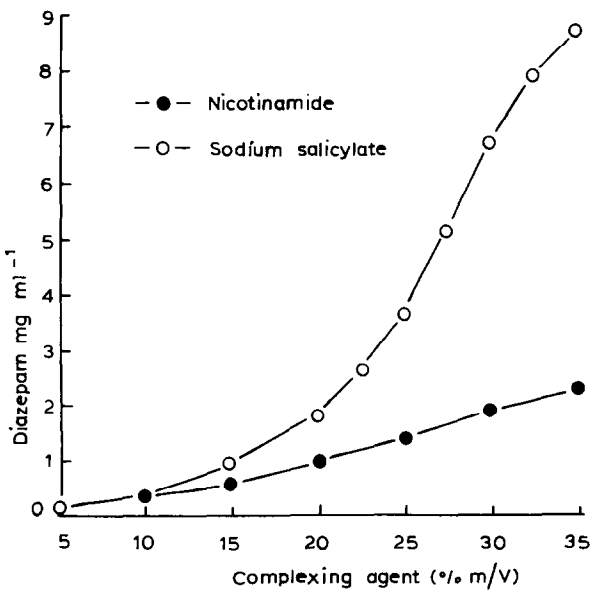

Fig. 2. Phase diagrams for the complexation of diazepam with sodium salicylate and nicotinamide.

increased structuring and hydrophobicity of water resulting from aggregation of high concentrations of the hydrotrope (Saleh et al., 1983).

Solutions of temazepam prepared using sodium salicylate as the complexing ligand indicated that up to 10 $mg \cdot ml^{-1}$ temazepam could be solubilised by a ligand concentration of 300 $mg \cdot ml^{-1}$ with no precipitation occurring upon refrigeration. However, such solutions developed an unacceptable deep yellow colouration within 1 week of storage at ambient temperature, and on sterilisation by autoclaving. Additionally, temazepam is perceived as having poor stability in aqueous solution (Launchbury, 1984). Therefore, the application of lyophilisation was considered necessary in addition to hydrotropic solubilisation so as to produce an aqueous temazepam formulation with an adequate shelf-life.

Initially a range of lyophilised temazepam formulations was produced (Table 1). A well-defined plug was achieved in all cases. Reconstitution, although rapid (about 15 s) yielded solutions with a slight opalescence, particularly when smaller volumes were used. The opalescence could be removed by filtering the reconstituted injection through a 0.22 μm filter. Overdrying was considered as the most likely cause of this problem and therefore both PVP and lactose were investigated as possible protective agents. A formulation containing sufficient lactose (Table 1) was found to give rapid reconstitution of the lyophilised plug to yield a clear solution. Subsequently, assessment of a parenteral formulation of temazepam solubilised in propylene glycol/water indicated that a dose of about 0.5 $mg \cdot kg^{-1}$ would be required to produce adequate sedation in man (Dundee, 1985). Reformulation to produce a lyophilised preparation containing 40 mg temazepam per vial was therefore necessary. This was achieved using sodium salicylate (400 $mg \cdot ml^{-1}$) and lactose (100 $mg \cdot ml^{-1}$). The lyophilised preparation remained colour free over 6 months at 40°C during which no loss of temazepam was detected. The formulation reconstituted rapidly to give a clear solution. Thus, a combination of hydrotropic solubilisation and subsequent lyophilisation yielded a parenteral temazepam formulation with an excellent expected shelf-life.

Since it may occasionally be necessary to dilute the reconstituted injection a range of possible diluents were investigated (Table 2). Dilution experiments on a range of formulations were conducted on the basis that temazepam in any diluted preparation should remain in solution for at least 2 h at room temperature. This is a necessary safety margin should an injection be reconstituted but not used immediately. The preferred formulation could be safely diluted 1 part in 2 with water or 1 part in 5 when 40% m/V propylene glycol was used as the diluent. Increasing the salicylate concentration to 450 $mg \cdot ml^{-1}$ allowed a 1 part in 4 dilution, though precipitation did occur in this case after about 45 min.

Initial assessment of the preferred lyophilised formulation was made in a rabbit model. Rapid and adequate sedation was produced. Recovery was normal with no apparent venous damage on follow-up. The overall results of this study suggest that a combination of hydrotropic solubilisation and lyophilisation may offer a suitable means to obtain a satisfactory parenteral formulation of temazepam.

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