International Journal of Pharmaceutics, 60 (1990) 35–39
Elsevier

IJP 02019

The effect of formulation on the plasma binding and blood/plasma concentration ratio of diazepam

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(Received 2 October 1989)
(Accepted 27 October 1989)

Key words: Diazepam; Plasma protein binding; Blood; Plasma ratio; Valium; Diazemuls; Mixed micelle

Summary

Blood was spiked with Valium injection, Diazemuls and a mixed micelle preparation of diazepam and the blood/plasma concentration ratios compared by ANOVA and multiple range testing. The lipid formulation was found to have a significantly higher blood/plasma ratio ($p < 0.05$) than the other two formulations. This suggests that a small amount of the Diazemuls formulation adheres to or is taken up by non-plasma constituents in blood. The plasma from the samples spiked with Valium had significantly higher free fractions of diazepam than the plasma from blood spiked with Diazemuls and the mixed micelle preparation. These results offer a possible explanation for the reported observation that larger doses of Diazemuls compared to Valium injection are required to produce equivalent levels of sedation.

Introduction

Diazepam was introduced as a parenteral preparation in the early 1960s by Roche Pharmaceuticals under the trade name 'Valium'. Because diazepam is a highly lipid-soluble drug and as such is insoluble in aqueous solutions, propylene glycol and ethanol are used as the solubilising agents. Since its introduction it has been widely used in clinical practice via the intravenous route. However, there have been many reports that diazepam in this formulation causes pain on injection and thrombophlebitis (Langdon et al., 1973; Graham et al., 1978; Kawar and Dundee, 1982). Various workers have suggested that the vehicle used to solubilise the diazepam (Table 1) is responsible for these sequelae (Keller, 1973; Graham et al., 1977). Subsequently, Kabi Vitrum introduced an emulsion formulation, Diazemuls (Table 1), which uses emulsified soya bean oil as a solvent for diazepam. This newer formulation has proved very successful in reducing the incidence of venous sequelae associated with intravenous administration of diazepam (Von Dardel et al., 1976; Olesen and Huttel, 1980; Selander et al., 1981; Kawar and Dundee, 1982). However, Gjessing and Tomlin (1977) noted that significantly larger doses of Diazemuls were required to produce the same level of sedation as Valium. This led to further pharmacokinetic investigations (Von Dardel et al., 1983; Naylor and Burlingham, 1985; Fee et al.,

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TABLE 1

Formulation quantities per ml of three parenteral preparations of diazepam

Valium		Diazemuls		Valium mixed micelles	
Diazepam	5 mg	Diazepam	5 mg	Diazepam	5 mg
Benzyl alcohol	15.70 mg	Fractionated soya bean oil	150 mg	Lecithin (e.g., phospholipen 100)	169.3 mg
Alcohol	84.60 mg	Acetylated monoglyceride	50 mg	Glycocholic acid	88.5 mg
Propylene glycol	414.60 mg	Fractionated egg phospholipids	12 mg	Sodium pyrosulphite pure	1 mg
Benzoic acid	2.12 mg	Glycerol	22.5 mg	NaOH 40%	19 µl
Sodium benzoate	48.8 mg	Sodium hydroxide to pH 8.0		Benzyl alcohol	15 µl
Water for injection	1 ml	Water for injection	1 ml	1 N HCl to pH 6.0	q.s.
				Water for injection	1 ml

1986) and in each case plasma diazepam concentrations were higher after Valium administration than after Diazemuls administration. This was reflected in the area under the curve values which were reduced with the emulsion preparation (Fee et al., 1986).

Similar results have been reported with other drugs solubilised in an emulsion formulation (Cummings et al., 1984; Glen and Hunter, 1984). Earlier studies by Thorn-Alquist (1977), measuring blood concentrations of diazepam rather than plasma concentrations, found no differences between the two formulations.

Since it is only the free (unbound) portion of the drug in plasma which is pharmacologically active the work reported here was undertaken to investigate the possibility that differences in clinical effect were due to the formulation influencing:

(1) the degree of diazepam binding to plasma proteins and,
(2) the diazepam blood/plasma ratio.

Although not commercially available, a mixed micelle formulation of diazepam (see Table 1) intended for intravenous use (Roche Pharmaceuticals) was also included in the study.

Materials and Methods

Preparations of blood and plasma samples

Sample volumes of 15–20 ml of blood were obtained by venepuncture from 11 healthy subjects aged between 20 and 35 years. Each sample was expelled into three heparinised blood collecting tubes to give three approximately equal aliquots of blood. Addition of 1 µl of Valium injection was made to one aliquot, 1 µl of Diazemuls to the second and 1 µl of the mixed micelle preparation to the third. The samples were mixed gently on a rotary mixer at 16 rpm for 30 min. An aliquot of each spiked sample was transferred to clean, labelled plastic vials and stored at −20 °C until analysed. The remainder of each sample was centrifuged for approx. 10 min at 3500 rpm and the plasma transferred to clean, labelled plastic vials and stored at −20 °C until analysis.

The binding of diazepam to plasma proteins was investigated in all 11 samples obtained. The blood/plasma ratio of diazepam was determined in six of the 11 samples selected at random.

Equilibrium dialysis

Equilibrium dialysis was carried out using a Dianorm (Diachema, Zürich) equilibrium dialysis system. The base and lid of the dialysis cell were separated by a semipermeable membrane with a molecular mass cut off of 12–14 kDa (Scientific Instruments Centre, London).

1 ml of plasma was pipetted into one side of the dialysis cell and a protein-free filtrate of pooled plasma was pipetted into the opposite side. The pH of both the plasma and protein-free filtrate were adjusted to 7.4 immediately before dialysis. Dialysis was carried out for 3 h in a 37 °C water bath with the cells rotating at 12 rpm.

The free fraction, expressed as a percentage, was calculated as follows:

$$\text{free fraction} = \frac{\text{concentration of diazepam in protein-free filtrate}}{\text{concentration of diazepam in plasma}} \times 100.$$

Analysis of blood and plasma samples

The concentration of diazepam in the blood and plasma samples was measured by extracting the drug into benzene followed by gas liquid chromatography with electron capture detection (Gamble et al., 1975). Flunitrazepam was used as the internal standard and the coefficient of variation of the method was 10.9% for a sample with a mean value of 11.4 ng ml^{-1} and 7.2% for a sample with a mean value of 1066 ng ml^{-1}. As there was considerable difference in the recovery of diazepam from plasma and blood (unpubl. data) separate standard curves were prepared in blood and plasma when the respective samples were being analysed.

TABLE 2

The free fraction of diazepam in plasma from blood spiked with Valium injection, Diazemuls and a mixed micelle preparation

Subject	Formulation	Plasma concentration after dialysis (ng ml^{-1})	Concentration in protein-free filtrate (ng ml^{-1})	Free fraction (%)
A	Valium	1109	8.8	0.79
	Diazemuls	1563	9.9	0.63
	Micelles	1681	11.0	0.65
B	Valium	1529	12.7	0.83
	Diazemuls	2050	13.8	0.67
	Micelles	2252	17.6	0.78
C	Valium	1496	15.4	1.03
	Diazemuls	2017	18.2	0.90
	Micelles	1765	16.0	0.91
D	Valium	2454	26.4	1.08
	Diazemuls	3261	24.2	0.74
	Micelles	2504	24.8	0.99
E	Valium	1429	23.8	1.67
	Diazemuls	1664	16.5	0.99
	Micelles	1782	12.7	0.71
F	Valium	1950	24.8	1.27
	Diazemuls	1714	16.0	0.93
	Micelles	1630	16.0	0.98
G	Valium	1029	14.5	1.40
	Diazemuls	1047	12.4	1.18
	Micelles	903	11.8	1.31
H	Valium	1126	11.7	1.04
	Diazemuls	1431	10.1	0.71
	Micelles	1366	10.2	0.80
J	Valium	570	7.9	1.26
	Diazemuls	823	8.4	1.02
	Micelles	807	9.3	1.17
K	Valium	920	9.8	1.07
	Diazemuls	1020	9.4	0.92
	Micelles	1020	10.0	0.98
L	Valium	1258	12.2	0.97
	Diazemuls	1436	10.5	0.73
	Micelles	1431	11.8	0.82

TABLE 3

Blood/plasma concentration ratios in blood spiked with Valium injection, Diazemuls and a mixed micelle preparation

Subject	Preparation	Blood (ng ml^{-1})	Plasma (ng ml^{-1})	Blood/Plasma
A	Valium	735	1303	0.56
	Diazemuls	928	1608	0.58
	Micelles	928	1608	0.58
B	Valium	893	1916	0.47
	Diazemuls	1208	2065	0.58
	Micelles	770	2285	0.38
C	Valium	823	1506	0.55
	Diazemuls	1260	2149	0.59
	Micelles	998	1811	0.55
D	Valium	1400	2595	0.54
	Diazemuls	1768	2962	0.60
	Micelles	1453	2655	0.57
E	Valium	840	1422	0.59
	Diazemuls	963	1557	0.62
	Micelles	1050	1743	0.60
F	Valium	1208	2082	0.58
	Diazemuls	1033	1591	0.65
	Micelles	963	1743	0.55

Results

The samples of blood spiked with Valium injection consistently gave higher plasma-free fractions of diazepam than the same samples spiked with either Diazemuls or the mixed micelle preparation (Table 2). When subjected to single-factor ANOVA, the differences between the three groups were found to be significant at the 1% level. Multiple range testing (Newman–Keuls) showed highly significant differences between the Valium and Diazemuls groups ($P < 0.001$) and the Valium and mixed micelle groups ($P < 0.05$).

The individual diazepam concentrations in blood and plasma are shown in Table 3. The blood/plasma concentration ratios were compared by ANOVA and significant differences were found to exist ($P < 0.05$). Multiple range testing

showed that the Diazemuls group had significantly greater blood/plasma concentration ratios than both the Valium group ($P < 0.05$) and the mixed micelles group ($P < 0.025$).

Discussion

The Diazemuls preparation is a fat emulsion similar to Intralipid and there is evidence that lipid emulsions act as an additional storage depot for benzodiazepines (Krieglstein et al., 1974), resulting in a decreased free fraction. Similarly in the mixed micelle preparation, the diazepam is bound within the individual micelles. Although in theory the micelles rapidly disintegrate after injection into the blood and release the active drug into the circulation, it has been shown that this micellar binding prevents some of the drug from crossing the semipermeable membrane of an equilibrium dialysis system (Short and Rhodes, 1972). Sodium benzoate, which is present in the Valium formulation (Table 1), has been shown to displace bilirubin from its binding sites (Schiff et al., 1971). Although the diazepam and bilirubin bind to completely different sites on the albumin molecule (Roosdorp et al., 1977), it is possible that sodium benzoate, or some other constituent of the Valium preparation, may displace the diazepam from its binding sites. A combination of these three factors may account for the significant differences observed between the plasma-binding values of diazepam in the three formulations.

Electron microscopy of blood after the infusion of lipid emulsions similar to the Diazemuls vehicle has shown that lipid droplets adhere to the surface of platelets and may occasionally become embedded in holes on the platelet surface or be engulfed by platelets (Hovig, 1970). Red cells have also been found to be covered by a surface-film of lipid after administration of fat emulsions (Branemark and Lindstrom, 1964). The results presented in Table 1 suggest that diazepam in the emulsion formulation has a greater affinity for the non-plasma constituents of blood than the other two formulations. Droplets of Diazemuls may adhere to or be engulfed by platelets; alternatively, they may coat the surface of red cells or interact in some way with other blood cells such a leucocytes. This would lead to a significant amount of diazepam being 'spun down' with the red cells and buffy layer upon centrifugation, leading to lower plasma levels after Diazemuls administration.

The higher plasma-free fraction of diazapam in samples spiked with Valium injection together with the greater affinity of Diazepam for non-plasma constituents of blood in samples spiked with Diazemuls offer possible explanations for the greater clinical potency of Valium injection over Diazemuls. Although there are no reports of the clinical potency of the mixed micelle preparation, the results reported here would suggest an efficacy intermediate between that of Valium injection and Diazemuls.

References

Branemark, P.I. and Lindstrom, J., Microcirculatory effects of emulsified fat infusions. Circ. Res., 15 (1964) 124–130.

Cummings, G.C., Dixon, J., Kay, N.H., Windsor, J.P.W., Major, E., Morgan, M., Sear, J.W., Spence, A.A. and Stephenson, D.K., Dose requirements of ICI 35 868 (Propofol 'Diprivan') in a new formulation for induction of anaesthesia Anaesthesia, 39 (1984) 1168–1171.

Fee, J.P.H., Collier, P.S. and Dundee, J.W., Bioavailability of three formulations of intravenous diazepam. Acta Anaesth. Scand., 30 (1986) 337–340.

Gamble, J.A.S., Assaf, R.A.E., McKay, J.S., Kennedy, M.S. and Howard, P.J., Estimation of plasma diazepam. Anaesthesia 30 (1975) 159–163.

Gjessing, J. and Tomlin, P.J., An unusual solvent. Br. J. Anaesth., 49 (1977) 954.

Glen, J.B. and Hunter, S.C., Pharmacology of an emulsion formulation of ICI 35 868. Br. J. Anaesth. 56 (1984) 617–626.

Graham, C.W., Pagano, R. and Conner, J.T., Pain and clinical thrombophlebitis following IV diazepam and lorazepam. Anaesthesia, 33 (1978) 188–191.

Graham, C.W., Pagano, R. and Katz, R.L., Thrombophlebitis after IV diazepam can be prevented. Anesth. Analg. Curr. Res., 56 (1977) 409.

Hovig, T., Influence of various compounds and surfaces on blood platelets and platelet aggregates. A scanning electron microscope study. Ser. Haematol., 3 (1970) 47–67.

Kawar, P. and Dundee, J.W., Frequency of pain on injection and venous sequelae following the i.v. administration of certain anaesthetics and sedatives. Br. J. Anaesth., 54 (1982) 935–939.

Keller, M.F., IV diazepam administration. J. Am. Med. Assoc., 225 (1973) 750.

Krieglstein, J., Meffert, A. and Niemeyer, D.H., Influence of emulsified fat on chlorpromazine availability in rabbit blood. Experientia, 30 (1974) 924–926.

Langdon, D., Harlan, J. and Bailey, R., Thrombophlebitis with diazepam used IV. J. Am. Med. Assoc., 223 (1973) 184.

Naylor, H.C. and Burlingham, A.N., Pharmacokinetics of diazepam emulsion. Lancet, i (1985) 518–519.

Olesen, A.S. and Huttel, M.S., Local reactions to IV diazepam in 3 different formulations. Br. J. Anaesth., 52 (1980) 609–611.

Roosdorp, N., Wann, B. and Sjoholm, I., Correlation between arginyl residue modification and benzodiazepine binding to human serum albumin. J. Biol. Chem., 252 (1977) 3876–3880.

Schiff, D., Chan, G. and Stern, L., Fixed drug combinations and the displacement of bilirubin from albumin. Pediatrics, 48 (1971) 139–141.

Selander, D., Curelaru, I., Stefansson, T., Local discomfort and thrombophlebitis following IV injection of diazepam. Acta Anaesth. Scand., 25 (1981) 516–518.

Short, M.P. and Rhodes, C.T., Effects of surfactants on diffusion of drugs across membrane. Nature New Biol., 236 (1972) 44–45.

Thorn-Alquist, A-M., Parenteral use of diazepam in emulsion formulation. A clinical study. Acta Anaesth. Scand., 21 (1977) 400–404.

Von Dardel, O., Mebius, C. and Mossberg, T., Diazepam in emulsion form for IV usage. Acta Anaesth. Scand., 20 (1976) 221–224.

Von Dardel, O., Mebius, C., Mossberg, T. and Svensson, B., Fat emulsion as a vehicle for diazepam; a study of 9492 patients. Br. J. Anaesth., 55 (1983) 41–47.