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**Research Papers**

# DIAZEPAM-SODIUM SALICYLATE SOLUTION: DILUTION WITH INTRAVENOUS FLUIDS, IN VITRO HAEMOLYTIC ACTIVITY AND PROTEIN BINDING

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## SUMMARY

The compatibility and stability of diazepam 5 mg/ml in 30% sodium salicylate, following dilution with 5% dextrose and normal saline, was investigated. Test dilutions ranging from 1 : 1 to 1 : 100 did not result in immediate precipitation and remained clear for at least 1 h and 3 h after dilution with 5% dextrose, normal saline or human plasma respectively. However, microcrystal precipitation was noted in some solutions thereafter.

No precipitation was observed when diazepam–sodium salicylate solution was injected at varying rates into the tubing of 5% dextrose and saline infusions moving at low rates.

Diazepam–sodium salicylate solution induced a higher degree of haemolysis in vitro and was less bound to bovine serum albumin than a commercial diazepam injection. Sodium salicylate competitively inhibited the binding of diazepam to bovine serum albumin.

Further studies are necessary for the clinical evaluation of this diazepam–sodium salicylate combination.

## INTRODUCTION

There is considerable controversy regarding the dilution of diazepam injection with propylene glycol as the main solvent. Failure of the vehicle to maintain complete solubilization of the drug after dilution resulted in the precipitation of diazepam in intravenous preparations (Jusko, 1973; Morris, 1978) and presumably at the injection site (Langdon et al., 1973; Assaf et al., 1975). This precipitation as well as the local irritation produced by propylene glycol could be responsible for the undesirable clinical effects reported after diazepam injection (Graham et al., 1977; Keller, 1973). However, diazepam infusion in intravenous fluids has been used in various cases with confusion concerning the stability and compatibility of the injection after dilution (Baxter et al., 1977; Tehrani and Cavanaugh, 1977) and the rate of infusion (Jusko et al., 1973; Korttila et al., 1976).

In a previous communication, we suggested the use of 30% sodium salicylate in water

as a solvent for diazepam solution, 5 mg/ml (Saleh et al., 1980). The present study was carried out to investigate the compatibility and stability of this diazepam solution following dilution with two intravenous fluids and plasma. The in vitro haemolytic activity and the extent of binding to bovine serum albumin for diazepam–sodium salicylate solution and a commercially available diazepam injection were compared. Further, the effect of sodium salicylate on the haemolytic activity and protein binding of diazepam was studied.

## MATERIALS AND METHODS

### *Dilution with intravenous fluids*

Test dilutions of diazepam [1] solution, 5 mg/ml, in 30% sodium salicylate [2] were prepared in thoroughly cleaned and dried volumetric flasks, with 5% dextrose [3], normal saline [4] or human plasma [5]. Dilutions were made in duplicate at an ambient temperature of 18°C. The prepared dilutions (1 : 1 to 1 : 100 corresponding to 250 mg/100 ml to 5 mg/100 ml) were examined with a reading lens for the presence of visible precipitate or microcrystals using a sample of the intravenous fluid for comparison.

Samples of the test dilutions with 5% dextrose and normal saline were suitably diluted with 0.1 N HCl and assayed spectrophotometrically [6] for diazepam content by measuring the extinction at 365 nm. At this wavelength, the highest concentration of sodium salicylate in the diluted samples did not interfere in the assay of diazepam. The percentage concentration of diazepam in solution was calculated with reference to the initial concentration.

The reported results are the means of two experiments.

In another experiment, diazepam–sodium salicylate solution was injected at rates ranging from 0.5 to 2 ml/min into infusion tubes containing 5% dextrose or normal saline moving at rates ranging from 5 to 20 ml/min and the solutions were inspected for precipitation visually. The pH values of the test solutions were measured [7] immediately and 24 h after dilution.

### *Hemolysis study*

Human blood [5] was used after defibrination. The RBCs were washed with normal saline and centrifuged until the supernatant was colourless. The RBCs were then diluted to the original volume with normal saline. Different concentrations of diazepam were prepared by diluting diazepam–sodium salicylate solution and a commercially available diazepam injection [8] with normal saline. A colorimetric method (Ansel and Cadwallader,

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[1] Courtesy of Hoffmann-La Roche Pharma Division, Basle, Switzerland.
[2] Riedel-De Haen AG, Seelze-Hannover, G.F.R.
[3] B. Braun, Melsungen AG, G.F.R.
[4] Vifor, S.A. Geneva, Switzerland.
[5] Blood Bank, Tripoli, Libya.
[6] Shimadzu Digital Double-Beam Spectrophotometer UV-150-02.
[7] Corning pH meter 113.
[8] Stesolid, 10 mg/2 ml, Lot. no. 47516, Dumex, Denmark.

1964) was employed to determine the degree of haemolysis in each test solution. One-tenth ml of RBCs suspension was incubated with 10 ml of the different solutions at 25°C for 45 min. The unhaemolyzed cells were settled by centrifugation at 3000 rpm for 10 min and the absorbance readings of the haemolysate determined at 550 nm. Each absorbance reading was compared with a total haemolysis reading obtained by laking red cells in distilled water. The degree of haemolysis occurring in each test solution was calculated as a per cent of total haemolysis. The data presented are the means of 3 experiments. Similar experiments were carried out to test the haemolytic activity of different concentrations of sodium salicylate.

*Protein binding study*

The binding of bovine serum albumin [9] of diazepam in sodium salicylate solution and in Stesolid was studied using the equilibrium dialysis method. Both solutions were diluted to 20 mg/100 ml diazepam with 0.15 M phosphate buffer, pH 7.4. The same buffer was used to prepare 1% BSA solution immediately before experimentation. Dialysis bags [10] filled with 10 ml of the albumin solution were placed in paired glass tubes containing 10 ml of the test solution. Equilibrium was established by mechanical shaking at room temperature (22°C) for 7 h, sufficient time for the dialysis equilibrium of diazepam. The concentration of free diazepam in the outer solution was determined spectrophotometrically at 365 nm. The reported per cent diazepam bound represents the mean of two determinations.

In a similar experiment, the effect of different concentrations of sodium salicylate on the per cent diazepam bound was studied. Further, the binding of diazepam ($5–20 \times 10^{-5}$ M) to BSA in the absence and the presence of sodium salicylate $2 \times 10^{-3}$ M was investigated. A molecular weight of 69,000 has been assumed for BSA.

## RESULTS AND DISCUSSIONS

*Dilution with intravenous fluids*

Dilution of diazepam, 5 mg/ml in 30% sodium salicylate, with 5% dextrose or normal saline did not result in immediate precipitation or transient cloudiness under all conditions of dilution. Table 1 shows that all test solutions remained clear for at least 1 h and 3 h after dilution with 5% dextrose and saline, respectively. Slight to clearly visible microcrystalline precipitate was noted in some dilutions while others did not show any sign of incompatibility 24 h after dilution.

The stability of diazepam in the test solutions was followed up by determining the drug concentration over a period of 24 h (Tables 2 and 3). Diazepam concentration remained unchanged except in solutions showing microcrystal formation.

When diazepam–sodium salicylate solution was diluted with plasma, no precipitation was noted in all dilutions during the 3-h study period. However, immediate precipitation was visible when Stesolid was diluted with plasma under the same conditions. Precipitation of diazepam injection in human plasma has also been reported by Jusko et al.

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[9] BSA min. 92%, Cohn, Fraction V.

[10] Sigma Chemicals, St. Louis, Mo. 63178 U.S.A.

TABLE 1

PRECIPITATION OF DIAZEPAM, 5 mg/ml IN 30% SODIUM SALICYLATE SOLUTION AFTER DILUTION WITH 5% DEXTROSE AND NORMAL SALINE

| Dilution | Time in hours | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 1.5 | 2 | 3 | 5 | 7 | 24 | 1 | 3 | 5 | 7 | 24 |
| | 5% Dextrose | | | | | | | Normal saline | | | | |
| 1 + 1 | – | – | – | ± | + | + | + | – | – | – | – | – |
| 1 + 2 | – | + | + | + | + | + | + | – | – | – | – | – |
| 1 + 5 | – | + | + | + | + | + | + | – | – | ± | ± | + |
| 1 + 10 | – | ± | ± | + | + | + | + | – | – | ± | + | + |
| 1 + 15 | – | – | – | ± | + | + | + | – | – | ± | ± | + |
| 1 + 20 | – | – | – | ± | + | + | + | – | – | – | – | + |
| 1 + 30 | – | – | – | – | – | + | + | – | – | – | – | ± |
| 1 + 50 | – | – | – | – | – | – | ± | – | – | – | – | ± |
| 1 + 100 | – | – | – | – | – | – | – | – | – | – | – | – |

– = no precipitation; ± = slight precipitation; + = clearly visible microcrystalline precipitation.

(1973). Further, no precipitation was observed when diazepam–sodium salicylate solution was injected into the tubing of running 5% dextrose or normal saline infusion at a rate of 2 ml/min even though the infusion rate was as low as 5 ml/min.

Lack of immediate precipitation following dilution of diazepam–sodium salicylate solution and after injecting this solution into running infusions can be attributed to the aqueous nature of the solvent system used. Such a system is likely to provide, upon dilution with aqueous fluids, a relatively stable supersaturated diazepam solution. This could be advantageous if one considers the clinical problems attributable to the precipitation of

TABLE 2

PER CENT * OF INITIAL CONCENTRATION OF DIAZEPAM, 5 mg/ml IN 30% SODIUM SALICYLATE, AFTER DILUTION WITH 5% DEXTROSE

| Dilution | 0.5 h | 2 h | 5 h | 7 h | 24 h | pH |
|---|---|---|---|---|---|---|
| 1 + 1 | 99.7 | 99.7 | 88.2 | 65.2 | 39.2 | 6.30 |
| 1 + 2 | 99.7 | 94.0 | 57.2 | 40.2 | 26.0 | 6.20 |
| 1 + 5 | 101.4 | 76.0 | 37.6 | 25.6 | 18.6 | 6.20 |
| 1 + 10 | 101.0 | 94.5 | 38.0 | 27.5 | 21.3 | 6.10 |
| 1 + 15 | 102.5 | 102.5 | 60.8 | 43.9 | 27.0 | 5.80 |
| 1 + 20 | 98.3 | 99.8 | 78.5 | 56.7 | 33.6 | 5.80 |
| 1 + 30 | 100.4 | 101.0 | 100.4 | 78.6 | 43.0 | 5.80 |
| 1 + 50 | 99.8 | 99.6 | 99.6 | 99.7 | 82.6 | 5.75 |
| 1 + 100 | 99.6 | 99.6 | 99.7 | 99.6 | 99.6 | 5.70 |

* The calculated values are the means of two determinations.

TABLE 3

PER CENT * OF INITIAL CONCENTRATION OF DIAZEPAM, 5 mg/ml IN 30% SODIUM SALICYLATE, AFTER DILUTION WITH NORMAL SALINE

| Dilution | 0.5 h | 2 h | 5 h | 7 h | 24 h | pH |
|---|---|---|---|---|---|---|
| 1 + 1 | 100.4 | 100.5 | 100.5 | 100.3 | 100.0 | 6.75 |
| 1 + 2 | 100.6 | 100.7 | 100.5 | 100.5 | 29.9 | 6.74 |
| 1 + 5 | 100.8 | 100.7 | 99.3 | 98.4 | 22.7 | 6.77 |
| 1 + 10 | 100.5 | 100.5 | 90.0 | 76.4 | 20.7 | 6.70 |
| 1 + 15 | 100.1 | 101.0 | 97.0 | 95.9 | 25.5 | 6.40 |
| 1 + 20 | 100.8 | 100.7 | 99.8 | 99.3 | 66.6 | 6.40 |
| 1 + 30 | 101.1 | 101.0 | 100.4 | 100.0 | 95.0 | 6.60 |
| 1 + 50 | 99.3 | 99.2 | 99.1 | 99.2 | 90.8 | 6.50 |
| 1 + 100 | 99.4 | 99.4 | 100.8 | 100.4 | 100.8 | 6.50 |

* The calculated values are the means of two determinations.

diazepam injection with propylene glycol as the main solvent in intravenous fluids (Jusko et al., 1973; Morris, 1978) and presumably in the veins after injection (Lingjaerde and Nordbø, 1974; Langdon et al., 1973).

The pH values of the test solutions are presented in Tables 2 and 3. They ranged from 5.70 to 6.75. Measurement of pH immediately and 24 h after dilution resulted in nearly constant values for all solutions. This slightly acidic pH would not affect the stability of diazepam which undergoes hydrolysis at high and low pH levels (King, 1973).

*Haemolytic activity*

The haemolysis curves obtained for diazepam–sodium salicylate solution and Stesolid are presented in Fig. 1. Both solutions were diluted to different concentrations (20–50 mg/100 ml) using saline. The figure shows that diazepam–sodium salicylate solution exerted a higher haemolytic effect. This could not be attributed to a difference in pH as the pH values of all solutions tested were in the range of 6.40–6.70. Various solvent systems were reported to alter the haemolytic response of the erythrocyte to chemical substances (Ansel, 1965; Ansel and Cabre, 1970). Therefore, the haemolytic effect of sodium salicylate solution was tested over a concentration range of 1–10% in saline. The haemolysis curve obtained (Fig. 2) shows that salicylate concentrations up to 5% had a negligible haemolytic effect. Haemolysis caused by higher concentrations may be the result of surface tension decrease (Saleh and York, 1978).

Lack of salicylate haemolytic activity in the concentration range used in the first experiment (Fig. 1) indicates that haemolysis by diazepam–sodium salicylate solution was mainly diazepam induced. Although the nature of the interaction drug–vehicle and erythrocyte was not investigated, results of this study indicated that the cellular activity of diazepam against the erythrocyte was different in sodium salicylate solution and Stesolid. The drug was shown to be more active or more available to the erythrocyte in the former solution. It is important to note that the haemolysis occurring in vitro in this study would not be likely to occur in vivo as the intravenously injected solution will be diluted in the total blood volume.

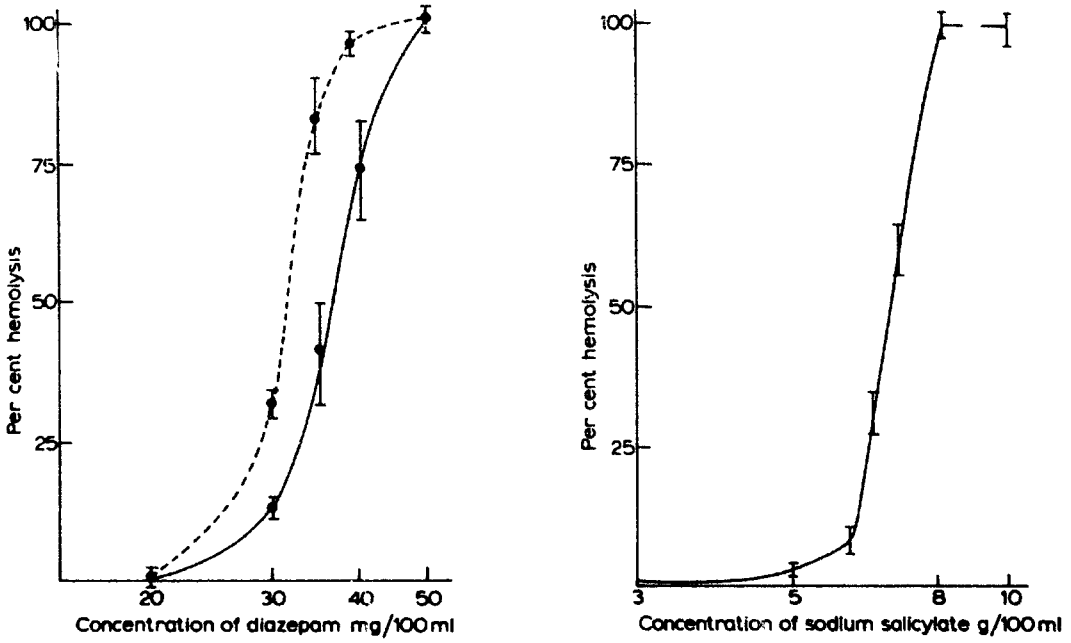


Fig. 1. Haemolytic activity of diazepam. – – –, diazepam–sodium salicylate solution in saline; ——, a commercial diazepam injection (Stesolid) in saline. Points represent the mean ± S.E. of the mean.

Fig. 2. Haemolytic activity of sodium salicylate in saline. Points represent the mean ± S.E. of the mean.

*Protein binding*

Results of the in vitro protein binding study indicated that diazepam in sodium salicylate solution was less bound (21.1 ± 1.8%) than diazepam in Stesolid (37.0 ± 1.0%). Salicylates are known to bind to serum albumin and displace many drugs from their binding sites (Sturman and Smith, 1967; Higashi et al., 1978). Thus, the effect of sodium salicylate on the binding of diazepam to bovine serum albumin (BSA) was studied. Increasing salicylate concentrations up to $0.8 \times 10^{-3}$ M markedly reduced the per cent of diazepam bound (Fig. 3). Higher salicylate concentrations resulted in the same inhibitory effect.

For further understanding of the nature of this inhibitory effect, the data obtained for the binding of diazepam to BSA in the absence and the presence of $2 \times 10^{-3}$ M sodium salicylate were plotted according to the Klotz equation (Klotz, 1953) and the results are shown in Fig. 4. Plotting of the reciprocal of the moles of diazepam bound per mole of albumin versus the reciprocal of the concentration of unbound diazepam resulted in straight lines with a common intercept. In the presence of salicylate less diazepam was bound to BSA. A common ordinate intercept indicates that diazepam and salicylate compete for the same binding site on albumin. It has been suggested that the benzodiazepine binding sites on BSA are located near positively charged groups of the protein molecule (Muller and Wollert, 1976). This is supported by our results which indicate that an

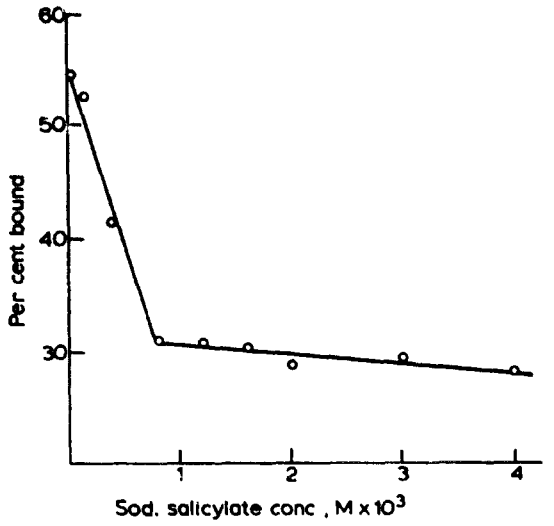


Fig. 3. Effect of increasing concentrations of sodium salicylate on the per cent diazepam bound to BSA.

anionic drug, sodium salicylate, known to interact electrostatically with cationic centres on albumin (Lindenbaum and Schubert, 1956) competes for diazepam binding sites on BSA.

In the present study, diluting diazepam 5 mg/ml in 30% sodium salicylate solution with 5% dextrose and normal saline did not result in immediate precipitation or transient cloudiness. However, microcrystal precipitation occurred in some test dilutions on storage at 18°C. This diazepam solution was injected at different rates into infusion tubes containing 5% dextrose or normal saline running at low rates without drug precipitation.

Diazepam in sodium salicylate solution exerted a higher haemolytic activity and was

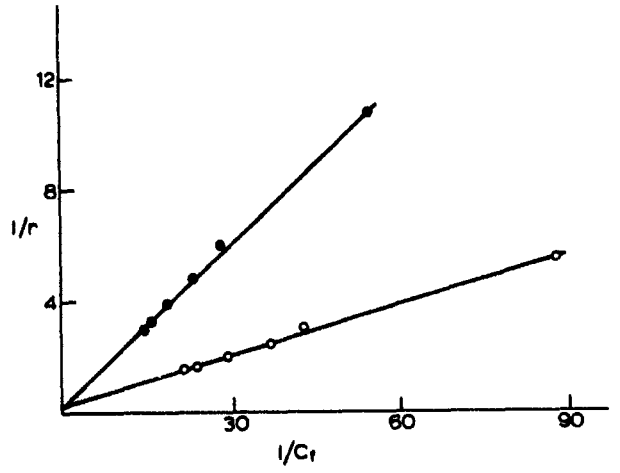


Fig. 4. Double-reciprocal plot for the binding of diazepam to BSA. ○, in the absence of salicylate, $r$ = 0.9978; ●, in the presence of $2 \times 10^{-3}$ M salicylate, $r$ = 0.9985. $r$ = correlation coefficient.

less bound to BSA compared to a commercially available diazepam injection (Stesolid). Sodium salicylate competitively inhibited the binding of diazepam to BSA.

Diazepam–sodium salicylate solution might be a clinically useful combination to achieve the tranquillizing and analgesic effects desirable in many instances. However, as the potency of benzodiazepines may be greatly modified by different solvent systems, either due to differences in solubilization of the drugs or to the pharmacologic activity of the solvent (Crankshaw and Raper, 1971), in vivo studies with diazepam–sodium salicylate solution will be undertaken.

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