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Report

Side-Effect Evaluation of a New Diazepam Formulation: Venous Sequela Reduction Following Intravenous (iv) Injection of a Diazepam Emulsion in Rabbits

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Diazepam has been incorporated into a stable, submicronized injectable emulsion. Venous sequela induction in rabbits following iv administration of diazepam in a marketed hydroalcoholic solution and in the emulsion were determined and compared over a 5-day period. There was a marked difference in the local reactions induced by the iv administration of the marketed diazepam hydroalcoholic solution and the diazepam emulsion, even on the first postinjection day. This difference was confirmed by pathological analysis. The highest mean venous sequela score was reached by the rabbit group injected with the marketed diazepam solution. It should be noted that no statistical difference was observed between the saline and the diazepam emulsion rabbit groups during the 5 days of the observation period. The moderate increase in the venous sequela score values compared to that for the saline solution should be attributed to the intrinsic effect produced by diazepam itself, and not to the emulsion vehicle, which was shown not to induce any vascular reaction in the present study.

KEY WORDS: diazepam; submicronized emulsion; venous sequelae.

INTRODUCTION

The currently marketed injectable products of benzodiazepines may induce thrombophlebitis following intravenous administration (1,2). Owing to their low aqueous solubility, diazepam and lorazepam injections are prepared by dissolving the benzodiazepines in organic solvent vehicles such as polyethylene glycol, propylene glycol, and ethanol. The toxic and side effects observed are attributed to these organic solvent vehicles. Administration of these benzodiazepine injections also results in pain at the injection site. Recently some authors have reported effective prevention of venous complications using a fat emulsion as the solvent for diazepam (3). Furthermore, the use of diazepam in the emulsion vehicle was investigated clinically by Von Dardel and colleagues (4). No significant difference could be demonstrated when compared with Valium injections but there was a marked reduction in pain at the site of injection. These results were recently confirmed (5).

Injectable diazepam has recently been formulated as a submicronized emulsion, the properties of which have been evaluated following the incorporation of another drug (6). Since this emulsion vehicle is also based on soybean oil, it is anticipated that the present modified emulsion vehicle will also be effective in preventing thrombophlebitis when administered intravenously. However, this hypothesis still remains to be proven.

The objective of the present study was to evaluate the venous sequelae of the diazepam emulsion compared to the local marketed diazepam when administered intravenously to rabbits.

MATERIALS AND METHODS

Materials

Purified soybean oil was purchased from Berlin Co. (Courbevoie, France). Diazepam was obtained from Teva Pharmaceutical Industries, (Kfar Saba, Israel) and meets the B.P. and U.S.P. requirements. The phospholipids were purified according to the method reported by Schubert and Wrethind (7). The marketed diazepam injections (Assival, equivalent to Valium injection, manufactured by Teva Pharmaceutical Industries, Kfar Saba, Israel, under license from Hoffman La Roche, Basel, Switzerland) were purchased from a retail pharmacy.

Emulsion Preparation and Evaluation

The basic method previously described was used (6). The phospholipids and diazepam were dissolved in the stabilized oily phase. The other excipients were dissolved in the aqueous phase. Both phases were heated separately to 70°C and dispersed by a magnetic stirrer. Emulsification was completed using a "high-speed mixer" for 5 min at 85°C. The resulting emulsion (1 liter) was cooled rapidly below 20°C and homogenized using a two-stage homogenizing valve assembly (Gaulin Homogenizer, APV Gaulin, Hilversum, The

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Netherlands) for 5 min. All the manufacturing procedures were carried out under aseptic conditions and a nitrogen atmosphere. The pH was adjusted to 7.4 using a sodium hydroxide solution (10%). The final fine emulsion was filtered to remove debris and coarse droplets and stored in ampoules. A typical formulation (%, w/w) consisted of the following: diazepam, 0.5%; oily phase, 20.0%; purified fractionated egg phospholipids, 1.2%; nonionic emulsifier, 2%; glycerin, 2.25%; α-tocopherol, 0.02%; methyl and butyl *p*-hydroxybenzoic ester, 0.3 and 0.075%, respectively; and water for injection, to 100.0 g. The emulsion vehicle was prepared using identical experimental conditions but without drug. This batch was duplicated and the zeta potential measured using the method previously described (8).

The mean and distribution particle size was measured using a photon correlator spectrometer, Malvern 4700 system (Malvern, Worcestershire, U.K.).

Stability Studies

Long-term stability studies were conducted at 4, 25, and 37°C. The chemical and physical changes that might occur in the emulsion during storage were followed up by visual observations (phase separation, creaming, etc.) and by measuring the various properties of the emulsions such as pH, zeta potential, and mean and distribution droplet size.

Venous Sequela Induction Evaluation

Twenty-two white rabbits (mixed strains) weighing 2.7–3.3 kg were used for this study. They were placed in a restraining hammock to facilitate cannulation. The degree of venous sequelae induced by the different solutions and emulsions was estimated and compared. The comparative evaluation was performed on the following preparations:

(a) marketed diazepam injection (0.5%, w/v),
(b) diazepam emulsion (0.5%, w/v),
(c) the emulsion vehicle (without diazepam), and
(d) saline solution.

The formulation of the marketed diazepam solution as reported in the PDR (9) consists of the following: propylene glycol, 40%; ethanol, 10%; benzyl alcohol, 1.5%; benzoic

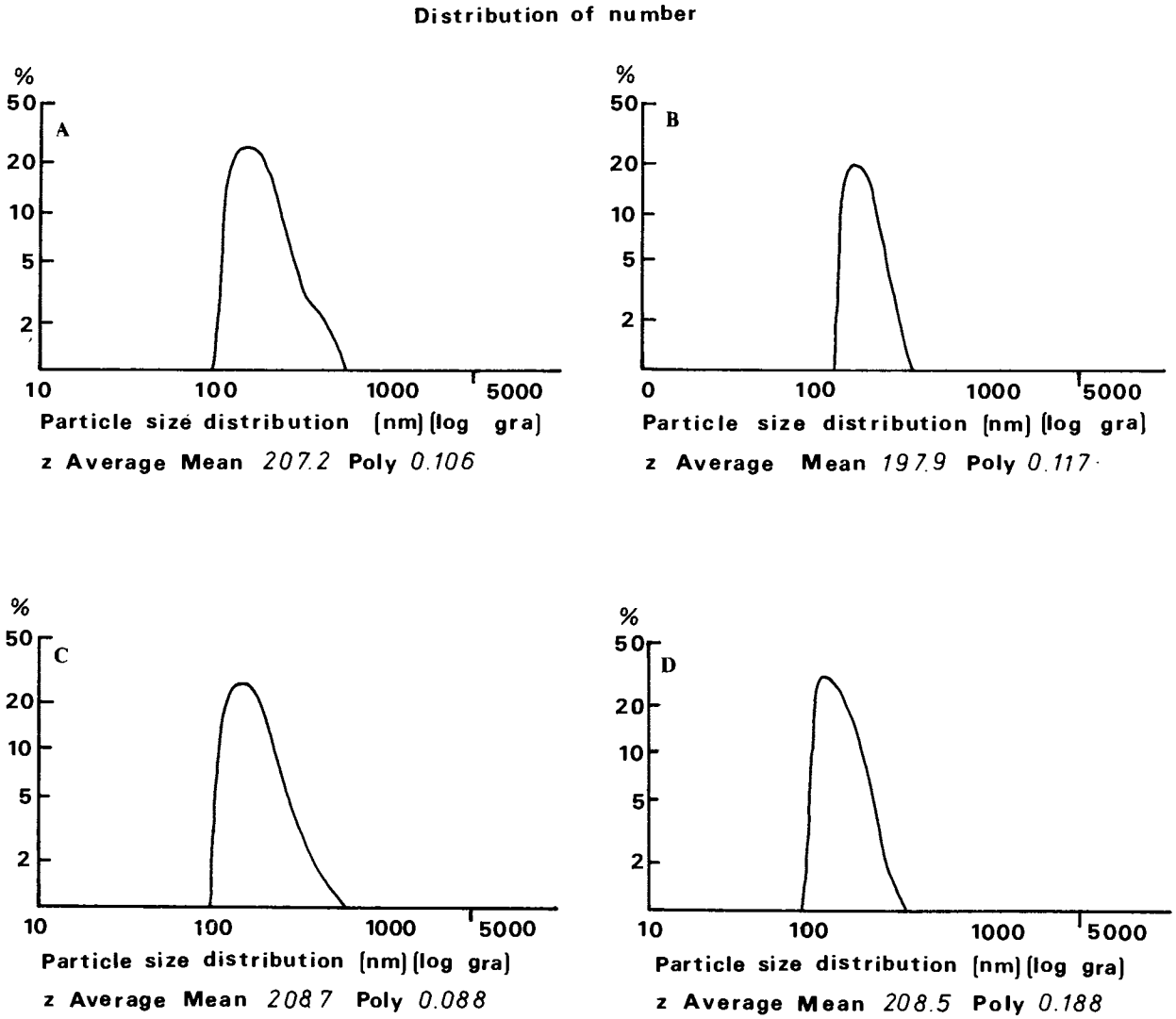

Fig. 1. Droplet size distribution profile of the diazepam emulsion just after preparation (A) and following 8 months of storage at 4°C (B), 20°C (C), and 37°C (D).

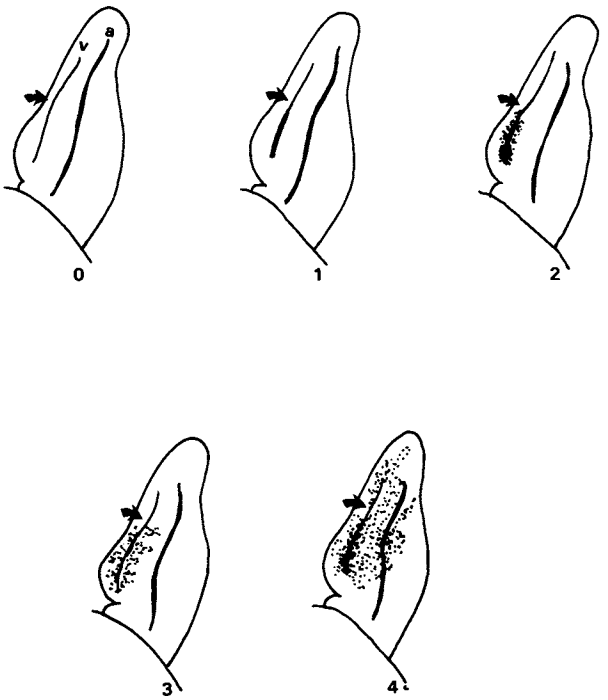

Fig. 2. Schematic illustration of rabbit ears of venous sequela score values of 0, 1, 2, 3, and 4. v, vein; a, artery. Arrows indicate the site of injection.

acid and sodium benzoate as buffers, 5%; and water, to 100ml. It should be emphasized that the marketed diazepam injection was a sterile preparation, whereas the diazepam emulsion and the emulsion vehicle were prepared under aseptic conditions and were free of microorganisms.

The 22 rabbits were divided into four groups. Three groups of six rabbits were injected iv with the diazepam injection, diazepam emulsion, and emulsion vehicle, respectively. The saline solution was injected iv to the fourth group, which comprises four rabbits.

A Venflon cannula N25 was inserted in the blood-flow direction, and the position of the cannula tip marked on the skin. The volume of the various preparations injected was 60 μl/kg, equivalent in the cases of the medicated preparations to the diazepam dose of 0.3 mg/kg per rabbit. An attempt was made to include rabbits weighing about 3 kg ± 10% in these groups, to prevent any variation in the volume of drug preparation administered.

The respective volume of drug or vehicle preparation was injected in the ear vein over 30 sec, the cannula was removed, and hemostasis was performed by light pressure of sterile gauze on the injection site. Visual evaluations (and photographs) of the area were done during 5 days following the injection. Local reactions were estimated on the basis of the results yielded by a preliminary pilot study.

The degree of venous sequelae was scored as follows:

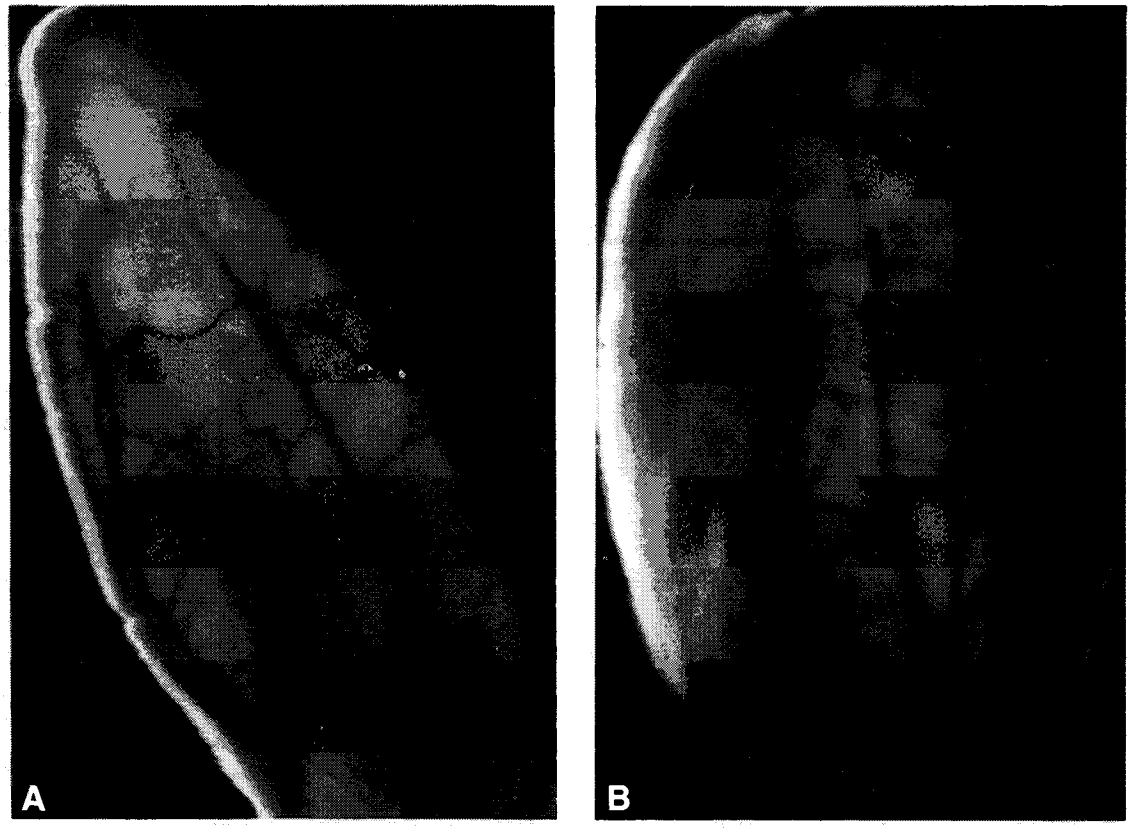

Fig. 3. Appearance of rabbit ears with venous sequela scores 1 (A) and 4 (B), 1 day after iv administration of emulsion vehicle and diazepam hydroalcoholic solution, respectively.

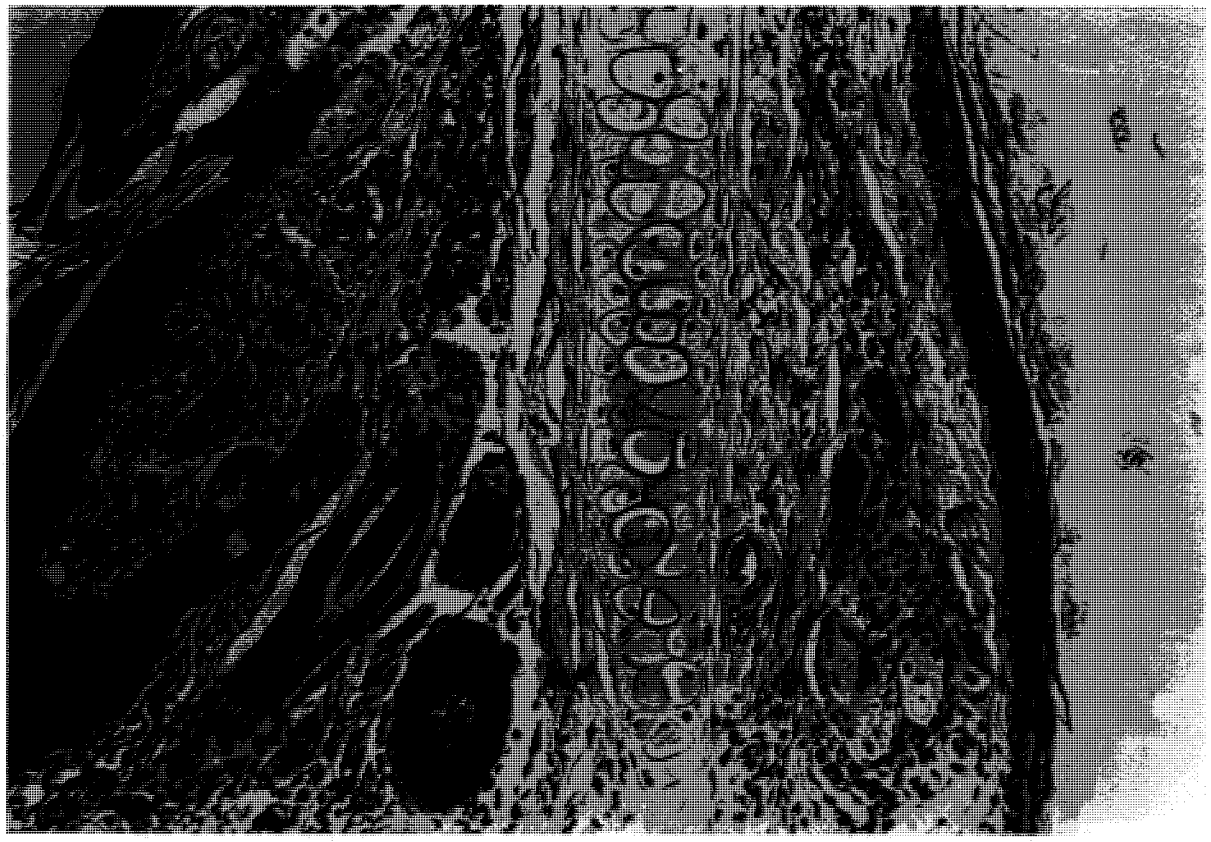

Fig. 4. Rabbit ear vein cross section typical of a venous sequela score value of 1–2 following iv administration of diazepam emulsion.

0 = no reaction,
1 = change in vein color without a significant reaction of the surrounding tissues,
2 = change in vein color and thickness accompanied by erythema or edema (limited to a 1- to 2-mm area transversing the vein), and
3 = edema and erythema of the vein and surrounding tissues (limited to a 5- to 8-mm area transversing the vein, for the length of the vein),
4 = inflammation of the ear over the area limited by the tissues surrounding the vein.

The total scores for venous sequelae of the groups of rabbits that received the various vehicles and diazepam preparations were pooled for each time point and analyzed statistically by the Wilcoxon rank sum *W* test.

Pathology Evaluation

In addition, following macroscopic evaluation of the local reaction according to the scale described above, samples of damaged tissues were carefully excised and subjected to pathological analysis. Analogical sections of intact ear animals were used as controls. An attempt was made to relate the statistical results of the visual observations with the pathological findings of the representative excised vein samples.

RESULTS AND DISCUSSION

Emulsion Dosage-Form Evaluation

The incorporation of drug into an emulsion normally may introduce a factor of instability, thereby inducing phase separation. It should then be emphasized that, prior to the animal experiments, an *in vitro* evaluation of the modified emulsion vehicle and corresponding diazepam emulsion was carried out.

Mean droplet size and zeta potential values of the emulsion vehicle and diazepam emulsion were −60 mV and 160 nm and −70 mV and 190 nm, respectively. These values indicated that diazepam incorporation did not affect the physicochemical properties of the stabilized emulsion. This was also confirmed by the results obtained during the long-term stability studies conducted on the diazepam emulsion. No significant change in zeta potential or pH was noted. Furthermore, no creaming at all was discerned over a period of 8 months' storage at different temperatures. In addition, no particle size increase was observed since no difference could be distinguished between the droplet size distribution profiles of the emulsions following 8 months of storage at various temperatures (Fig. 1). The low values of the mean droplet size reflected the formation of a close-packed mixed film of both emulsifying agents at the oil–water interface. It should be noted that the resulting zeta potential values were high enough to prevent coalescence of the droplets and preserved the integrity of the emulsion formed.

Venous Sequela Evaluation

Pain and venous sequelae associated with iv administration of a hydroalcoholic solution of diazepam are reported at

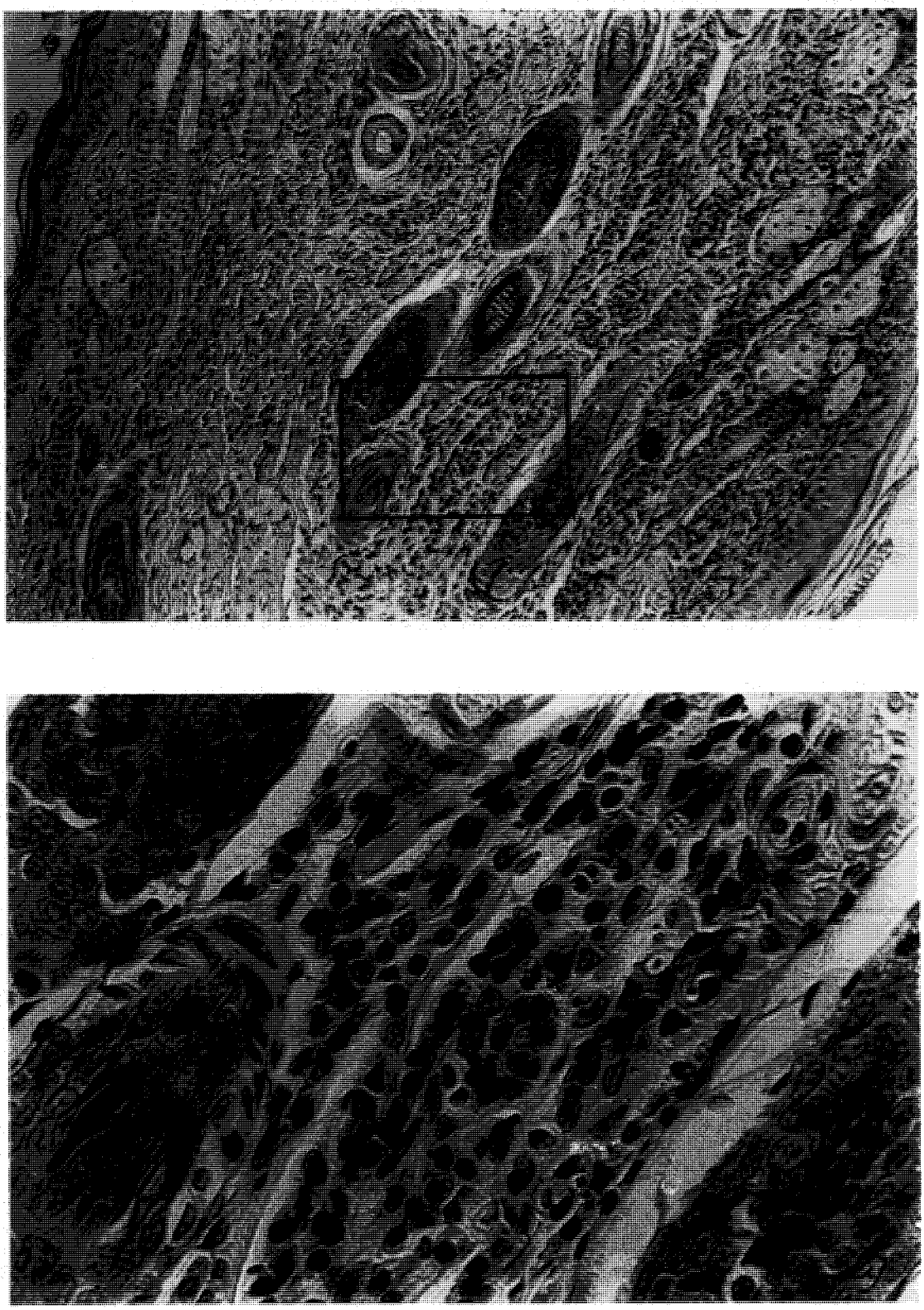

Fig. 5. Rabbit ear vein cross section typical of a venous sequela score value of 3–4 following iv administration of diazepam hydroalcoholic solution.

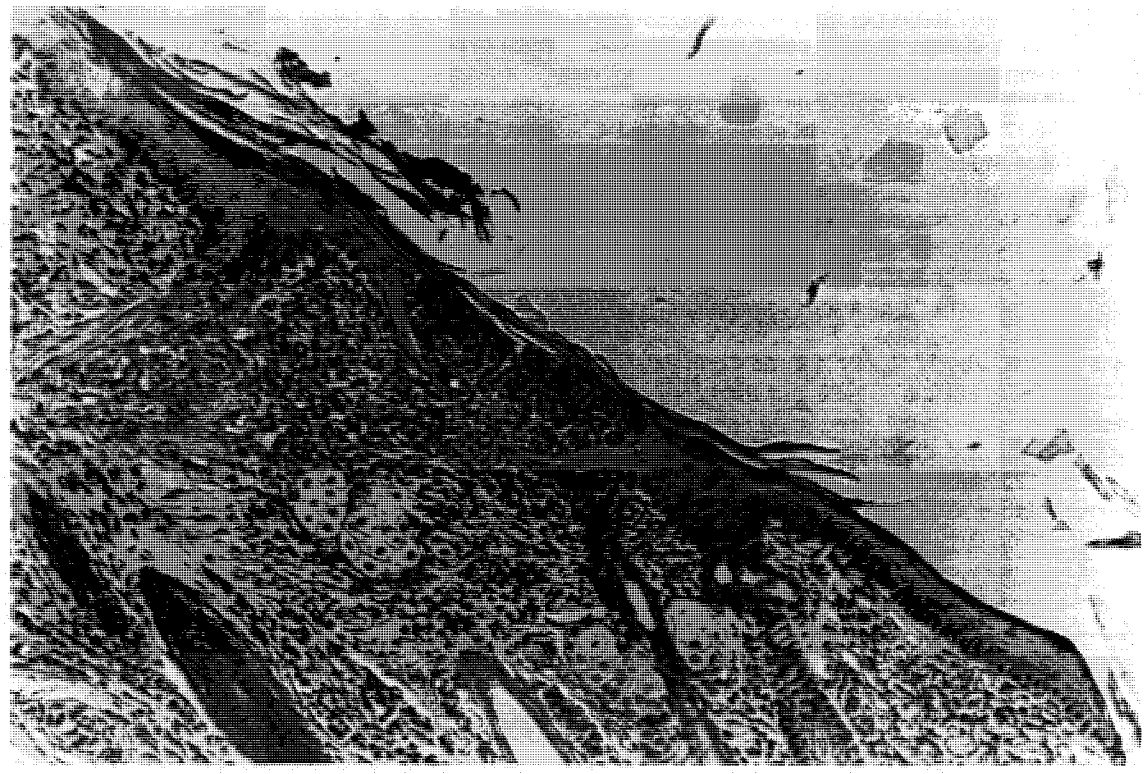

Fig. 6. Rabbit ear vein cross section following iv administration of diazepam hydroalcoholic solution showing microabscesses which accompanied a venous sequela score of 3–4.

a relatively high frequency. Both side effects can be prevented by using a diazepam formulation in which the drug is dissolved in the oil phase of an emulsion. Kronevi and Ljungberg (10) have already shown that, following a single administration (1 ml) in the central ear artery in rabbits, the hydroalcoholic formulation caused severe distal inflammation and necrosis. Furthermore, they have reported that the emulsion formulation (Diazemuls) in an equal volume and dose produces negligible morphological changes. This result is in accordance with the absence of venous sequelae following clinical use of intravenous Diazemuls (11). In the present study, the various diazepam dosage forms and vehicles were injected iv using a smaller volume of preparation (about 0.18 ml) than that injected by Kronevi and Ljungberg (10).

No difference was observed between the rabbit group injected with the stabilized emulsion vehicle and the group injected with saline. In both rabbit groups, no significant

Table I. The Mean Score for Venous Sequela Reaction Induced by the Different Diazepam Dosage Forms and Saline in Rabbits: Maximum Severity Score Value = 4[a]

Time (days)	Saline[b]	Diazepam emulsion (0.5%, w/v)[c]	Diazepam hydroalcoholic solution (0.5%, w/v)[c]
1	0.75 ± 0.5*	1.16 ± 0.98	2.33 ± 1.03
2	0.5 ± 0.57**	1.16 ± 0.40**	2.5 ± 0.54
3	0.5 ± 0.57**	0.83 ± 0.40**	2.66 ± 0.81
4	0.25 ± 0.50*	0.66 ± 0.51**	2.0 ± 0.63
5	0 ± 0**	0.50 ± 0.54*	1.83 ± 0.75

[a] No statistical difference was observed between the saline and the diazepam emulsion rabbit groups during the 5-day period of the experiment.
[b] Mean of four rabbits.
[c] Mean of six rabbits.
* Significantly different from values for hydroalcoholic diazepam solution using Wilcoxon rank sum test; $P < 0.05$.
** Significantly different from values for hydroalcoholic diazepam solution using Wilcoxon rank sum test; $P < 0.01$.

reaction surrounding the tissues at the site of injection was detected, although a change in vein color was observed. Schematic representations of rabbit ears prior to and 1 day following iv administration of saline and emulsion vehicle are shown in Fig. 2 at venous sequela scores of 0 and 1, respectively. These moderate effects did not persist during the observation period and the change in vein color disappeared 3 days after the injection in the majority of the rabbits of both groups.

The schematic representations (Fig. 2) reflect with accuracy the actual findings of the various experiments as can be deduced from the photographs in Fig. 3, where rabbit ears are shown at venous sequela scores of 1 and 4 one day after iv administration of emulsion vehicle and marketed diazepam, respectively.

There was a marked difference in the local reactions induced by the iv administration of the marketed diazepam hydroalcoholic solution and the diazepam emulsion, even on the first postinjection day. The ears in which the diazepam emulsion was injected were slightly swollen. The maximum venous sequela score reached in two rabbits was 2. Most of the rabbit ears treated with diazepam emulsion were free from abnormal signs. Local hematomas around the injection sites without edema were recorded and, in most cases, assigned the score of 1. Pathological analysis revealed the existence of a neutrophilic infiltration from both parts of the cartilage (Fig. 4). However, a more prominent infiltration could be clearly noted at the site of injection. These moderate reactions were detected mainly on the first postinjection day. They diminished progressively and almost disappeared on the fifth postinjection day. In contrast, the rabbit ears injected with the marketed aqueous diazepam solution were rapidly erythematose and edematose (venous sequela score of 2). These effects became progressively worse, reaching a maximum on the third postinjection day, and persisted over the entire observation period. Furthermore, erythema, edema, and inflammatory reactions spreading all over the vein section were observed in most of the rabbit ears in this group, reaching scores of 3 and 4, as illustrated in Fig. 3B.

The pathological findings characterizing severe inflammation associated with venous sequela scores of 3 and 4 are shown in Figs. 5 and 6. One notices that a massive neutrophilic infiltration over the entire area had occurred. This infiltration was also accompanied by microabscesses comprising pycnotic neutrophils in the epidermis (Fig. 6) and plasma-cell aggregates in the dermis as exemplified by Fig. 5. It should be noted that the pathological difference between a venous sequela score of 3 and a score of 4 was qualitative and quantitative. Regardless of the severity of the inflammation, in all the pathological observations including the control observations, a marginal neutrophilic infiltration was noted.

It could therefore be deduced that the various internal neutrophilic infiltrations observed were definitely associated with the administration of the various dosage forms. However, no thrombosis of blood vessels or inflammation around the vessel wall was found. It was therefore decided to define the adverse reactions as local inflammation or venous sequelae rather than thrombophlebitis.

In addition, the mean score for venous sequela reaction induced by the different diazepam dosage forms and the saline solution in rabbit ears was calculated for each time point over the 5 consecutive days of the observation period. The mean score results, which are reported in Table I, were compared using the Wilcoxon rank sum test. It can be seen from Table I that the highest mean score value was reached by the rabbit group injected with marketed diazepam solution, 2.66 ± 0.81, close to the maximum severity score value of 4. All the venous sequela score values of the aqueous diazepam group were significantly higher than the score values reached by the saline and diazepam emulsion groups.

It should be noted that no statistical difference was observed between the saline and the diazepam emulsion rabbit groups during the 5 days of the observation period.

This study confirmed previous results already reported in the literature that aqueous formulations of diazepam have demonstrated local vascular reaction after intravenous injections (4,11). These effects could be attributed either to the drug itself or to the solvents used. As reported, it could be that the cosolvents used to dissolve diazepam in the aqueous formulation are irritants to the vascular endothelium when their concentrations are high or that the drug precipitates locally at the site of the injected vein as a result of formulation dilution in the bloodstream, which reduced drastically its solubility in the microenvironment (12). These adverse effects could also be due to both types of mechanisms. The inflammation seen with this new injectable diazepam emulsion was restricted to the injection site, and the surrounding tissues were hardly affected. This should be attributed to the emulsion formulation, which comprised safe and pure components, and probably to the retention capacity of the tiny oil droplets, which should prevent the local precipitation of diazepam following immediate dilution in the bloodstream without affecting the expected rapid pharmacological onset.

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