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Research paper

Bioavailability of diazepam from aqueous-organic solution, submicron emulsion and solid lipid nanoparticles after rectal administration in rabbits ☆

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Abstract

The bioavailability of diazepam in rabbits after rectal administration of three formulations: organic-aqueous Relsed rectal solution (containing ethanol, benzyl alcohol and propylene glycol), submicron emulsion and solid lipid nanoparticles (SLN), was studied. Submicron emulsion contained MCT oil (20% w/w), egg lecithin and poloxamer; SLN were prepared with cetyl palmitate 10% w/v and non-ionic emulsifying agent, Plantacare. All formulations contained 4 mg/ml of diazepam and the dose administrated to rabbits was 2 mg/kg. In both submicron preparations nearly the same mean size of the dispersed particles (201–206 nm) and the fraction of the free drug in aqueous phase (0.9–1.5%) was determined. Besides very moderate prolongation of drug release, the submicron emulsion as a vehicle did not alter pharmacokinetics of diazepam when compared with the solution: the mean C_{max} was 48.9 ± 24.0 and 49.5 ± 17.0 ng/ml, and area under the curve was 134.0 ± 42.3 and 186.8 ± 59.8 ng h/ml, for solution and emulsion, respectively. The low relative bioavailability, 47% compared to the solution, was observed after administration of SLN. Transmission electron microscopy pictures revealed that some of diazepam is present on the surface of the SLN and this fraction was immediately absorbed, while the diffusion of the drug in the solid core was not efficient enough to allow a complete release. It may be concluded that submicron emulsion may be a good choice of an ethanol-free drug formulation, but lipid matrix, which is solid at body temperature, is not advantageous system for diazepam rectal delivery, even if delivered as a submicron dispersion.

Keywords: Diazepam; Rectal delivery; Submicron emulsion; Solid lipid nanoparticle, Liposphere; Pharmacokinetics

1. Introduction

Generally, oral administration of diazepam is the route of choice in the daily practice of pharmacotherapy. However, in some circumstances, when rapid sedative action is necessary or in the case of convulsions, the use of parenteral or rectal preparations is required. Rectal delivery of the drug is frequently used in some countries and especially appreciated in pediatric patients due to easy application also in treating convulsions [1–3]. This route of administration yields diazepam plasma levels and therapeutic efficacy comparable to that attained when the same dose is given orally or intramuscularly [3–5]. Using rectal solutions, fast absorption is achieved with peak plasma concentrations after only 15–20 min, while absorption from suppositories is delayed. Since diazepam is only very slightly soluble in water the solution must contain organic solvents: ethanol, propylene glycol and benzyl alcohol are present in the commercial preparations. Among those, ethanol is the least desirable component of pediatric drugs, however its concentration in diazepam rectal solutions is relatively high, i.e. 10% v/v.

Attempts to eliminate organic solvents in parenteral diazepam solutions resulted in a new formulation: submicron O/W emulsion. Diazepam-Lipuro (Braun), Stesolid (Dumex) and Dizac (Ohmeda) are examples of diazepam injectable emulsions available on the market. This delivery system originates from intravenous fat emulsions used for

☆ This work is dedicated to Professor Dr Dr h.c. Peter Pflegel, Greifswald, on the occasion of his 65th birthday.

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parenteral nutrition. Diazepam is dissolved in an oleaginous phase of the emulsion, with droplet mean sizes of approximately 300–400 nm. Components of the emulsion, natural oils and lecithin, make the preparation non-toxic and biocompatible. Submicron emulsions, primarily intended for parenteral application nowadays are also investigated for other routes of delivery like transdermal, oral or ocular [6–9]. Our studies focus on the use of such a system as a vehicle for rectal drug delivery. An emulsion containing diazepam, with parabens as preservatives, was prepared and the stability of the system was confirmed [10]. Solid lipid nanoparticles (SLN) are very much alike submicron emulsions, differing however in lipid nature since lipids which are solid in room temperature are used for this preparation [11,12]. SLN containing diazepam were prepared using lecithin as the emulsifying and stabilizing agent [13]; however, better physical stability was achieved using other non-ionic surfactants as poloxamer or alkyl glycosides.

The aim of our present study is the bioavailability of diazepam in rabbits after rectal administration of three formulations: organic-aqueous solution, submicron emulsion and SLN. Drug absorption from a two-phase vehicle may be different than from a solution, especially when solid matrix is present. It is, however, impossible to predict how the rectal absorption is influenced by organic solvents or surfactants. The formulation, which exhibits comparable to the commercial solution or even higher bioavailability and prolonged action, may be a candidate for future clinical studies.

2. Materials and methods

2.1. Materials

Diazepam was a gift from Polfa (Warsaw, Poland). Lipoid E-80 (egg lecithin) was purchased from Lipoid KG (Ludwigshafen, Germany) and Miglyol 812 (MCT oil) from Caelo Caesae and Loretz (Hilden, Germany). Synperonic F68 (poloxamer) was obtained from Boehringer Ingelheim (Heidelberg, Germany). Cetyl palmitate was obtained from Merck ((Darmstadt, Germany) and Plantacare 2000 (alkyl glucoside) was provided by Henkel (Düsseldorf, Germany).

Clonazepam used as an internal standard was a gift from Polfa (Tarchomin, Poland). Acetonitrile and hexane were obtained from Merck (Darmstadt, Germany) and methanol, sodium carbonate and potassium dihydrophosphate were from POCh (Lublin, Poland).

2.2. Formulations under investigation

All formulations contained 4 mg/ml of diazepam.

Submicron emulsion contained 20% MCT oil, 1.2% egg lecithin (Lipoid E-80), 2.0% poloxamer, 1.8% glycerol, 0.02% α-tocopherol as antioxidant and parabens (0.18% paraben M and 0.02% paraben P) as antimicrobial preservatives (w/v ratios). MCT oil was heated at 70°C and diazepam, parabens and tocopherol were dissolved. The lipid phase was added to an aqueous phase composed of water, poloxamer and glycerol, stirred at 70°C. The primary emulsion was stirred using a high-shear mixer (Ultra-Turrax, Janke and Kunkel, Staufen, Germany) at 20 500 rev./min for 10 min. The submicron dispersion was obtained by passing the emulsion through a high-pressure homogenizer (APV Gaulin, Hilversum, Holland) at 500 bar pressure (eight cycles). Adjustment of pH to 8.0 was done with 0.1 mol/l NaOH. The emulsion was filtered aseptically through Durapore filter (Millipore, Bedford, USA) and packed in sterile glass vials under nitrogen.

The SLN were prepared with cetyl palmitate 10% w/v and non-ionic emulsifying agent, Plantacare 1.2% w/v using a hot homogenization technique [14]. Diazepam was dissolved in the melted lipid at 64°C. The lipid phase was added to an aqueous solution of the surfactant at identical temperature. The mixture was stirred for 1 min with an Ultra-Turrax at 9500 rev./min and homogenized using a high-pressure homogenizer APV LAB 40 (Lübeck, Germany) at 500 bar (three cycles). The pH of SLN dispersion was 7.7 and was not adjusted.

Relsed rectal solution (manufactured by Polfa, Warsaw, Poland) was used as a reference preparation. The solution contained 10% ethanol, 1.5% benzyl alcohol, 40% propylene glycol (w/v ratios) as well as sodium benzoate and acetic acid.

Particle size analysis of SLN and submicron emulsion was performed using a photon correlation spectrometer Zetasizer 3000 (Malvern Instruments, Malvern, UK) and the Contin program was applied for calculations. Diazepam distribution between the oily and aqueous phase was also measured. In order to obtain an aqueous phase, the submicron emulsion or SLN were centrifuged and filtered through Microcon-100 (100 000 NMWL) centrifugal filtration unit (Millipore, Bedford, USA) and the ultrafiltrate was analyzed for diazepam concentration using a high-performance liquid chromatography (HPLC) method. Moreover, the concentration of diazepam was also determined in a supernatant obtained by a prolonged ultracentrifugation of the preparations. The ultracentrifugation was performed at a velocity 40 000 rev./min for 16 h using an ultracentrifuge Sorval combi plus (Du Pont Instruments, Wilmington, USA).

The electron microscopy of SLN was performed on a transmission electron microscope JEOL-1210 (Japan) at 120 keV. The system was diluted with bi-distilled water (1:10) before adsorption on a carbon-coated grids, and 1% uranyl acetate was used for the contrast.

2.3. Bioavailability studies

Six New Zealand rabbits weighing 3.6 ± 0.4 kg were used. The experiment was carried out after approval of the protocols by the ethical-scientific committee of the Medical University of Gdansk. The animals were kept in individual

cages, fed a standard diet and made to fast during 15 h prior to the experiment. Diazepam preparations were administered rectally using a syringe with a 6-cm long applicator. The dose administered was 2 mg/kg. Each animal received three formulations at 1-week intervals. Blood samples were collected from the marginal ear vein at 0, 20, 40, 60, 90 min, 2, 4, 6, 8 and 24 h after drug application. The blood was immediately mixed with 3.8% sodium citrate (1:9) and plasma was obtained by centrifugation of the blood at 4000 rev./min for 10 min in a Hermle Z centrifuge (Germany). The plasma was frozen at −30°C until analysis.

2.4. Determination of plasma diazepam concentration

Approximately 1 ml of plasma was spiked with 50 μl of the internal standard solution (10 μg/ml) and 400 μl of 10% sodium carbonate solution was added. The mixture was extracted with 5 ml of hexane and methanol (7:3 volume ratio). After 5 min centrifugation (3500 rev./min) the samples were frozen at −28°C, the organic layer was collected and evaporated. The dry residue was dissolved in 200 μl of the mobile phase and injected to the HPLC column.

Concentrations of diazepam in the samples were determined using a HPLC apparatus (Merck-Hitachi, Darmstadt, Germany) equipped with a 25 × 4-mm RP C-18 column (Lichrospher, 5 μm, Merck) and C-18 precolumn. The mobile phase was 0.5 mol/l potassium dihydrophosphate/water/acetonitrile (1.5:38.5:60). The flow rate was set at 1.0 ml/min, the volume of the injected sample was 20 μl and the detector wavelength was 254 nm. Under these conditions the retention times were 7.6 min for diazepam and 4.6 min for clonazepam.

The mean diazepam plasma recovery was 91.8 ± 11.9%, while 91.6 ± 10.1% of the internal standard was recovered. The calibration curve prepared with plasma spiked with standard solutions and extracted as described above was linear ($r = 0.9986$) in the 5–200 ng/ml range. The between-days precision was about 15% at the quantitation limit (4 ng/ml) and less than 7% at higher concentrations.

2.5. Pharmacokinetic analysis

The C_{max}, T_{max} and area under the curve $(AUC)_{0-24}$ were determined from individual plasma concentrations. The AUC was calculated by the trapezoidal rule without extrapolation to infinity. The pharmacokinetic parameters for different formulations were compared using the Student's *t*-test. The drug concentration–time profiles between each formulation were compared using one-way analysis of variance. Significance level was considered to be $P < 0.05$.

3. Results and discussion

Three different liquid formulations containing diazepam were administered rectally to rabbits. Concentration of diazepam in all preparations was identical, i.e. 4 mg/ml. Except for the solution two other formulations consist of two phases: aqueous and lipid. Distribution of diazepam between these phases was studied using ultrafiltration technique and it was found that 0.9 and 1.5% of the drug was present in the aqueous phase of submicron emulsion and SLN, respectively, and the rest was situated in the internal lipid phase and in the interface. After prolonged centrifugation of SLN 17% of the total content of diazepam was found in the supernatant, while 28% was determined for submicron emulsion. The drug found in the supernatant probably represents the portion located in the interface. No drug was found as a precipitate when the preparations were inspected visually.

Fig. 1 presents size distribution profiles of the lipid particles in both systems. All SLN particles were below 800 nm, while 20% of the oily droplets in the submicron emulsion were in the range 800–1200 nm. However, no difference in the mean volume weighted size of the dispersed particles was observed: the value was 206 nm and 201 nm for SLN and submicron emulsion, respectively. The lipid used for SLN preparation melts at 54°C. Thus, the two submicron dispersions may be characterized as systems where the drug is dissolved in the microcompartments which are similar in size; however, the matrix is solid or liquid.

Nearly 2 ml of the formulation had to be introduced rectally in order to deliver a 2 mg/kg dose of diazepam. A deep application to the rectum was necessary in order to avoid leakage of the preparation. The animals were carefully observed during the experiment. The response to the drug was fast: after only 5–10 min, sedation of the animals and muscle relaxation was observed. Intensity of the reaction varied from animal to animal. The animals were alert again between 30 min and 2 h after administration. The shortest reaction was in animals receiving SLN, while in all animals receiving emulsion, the effect lasted approximately 2 h.

Diazepam in blood was analyzed using the HPLC technique following extraction and concentration procedure. The bioavailability experiment was terminated after 24 h

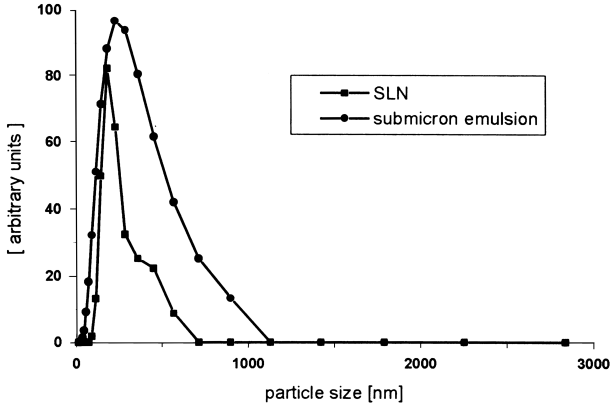

Fig. 1. Particle size distribution in diazepam submicron systems.

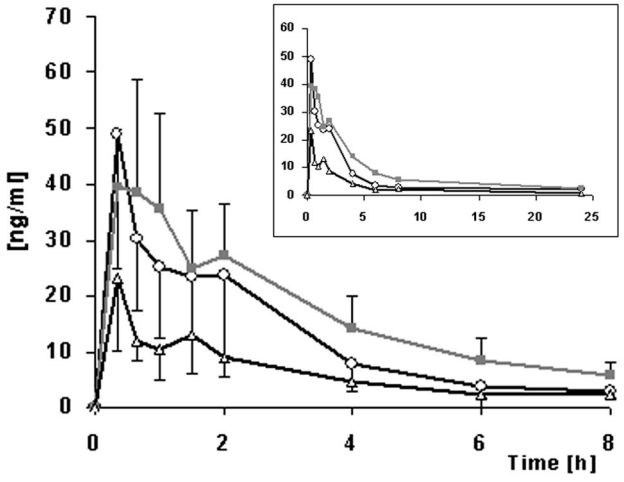

Fig. 2. Mean plasma concentration-time profiles (±SD, $n = 6$) after rectal administration of diazepam as submicron emulsion (squares), SLN preparation (triangles) and aqueous-organic solution (Relsed) (circles).

although $t_{0.5}$ for diazepam in humans is 20–40 h [4]. The preliminary experiments revealed that in rabbits no peaks of diazepam were observed on chromatograms of blood samples collected after 48 h.

Fig. 2 presents plasma concentration–time profiles of diazepam and the pharmacokinetic parameters are shown in Table 1. No significant difference is noticed between C_{max} and AUC_{0-24} values when the drug was administered either in aqueous-organic solution or submicron emulsion. The mean T_{max} calculated from individual T_{max} values is longer in animals receiving emulsion (Table 1) but the difference occurred only in four out of six animals and the mean plasma profile does not show such delay of absorption (Fig. 2). For both formulations diazepam concentrations measured after 20 min were in the range 30–66 ng/ml in all animals, although one concentration of 90 ng/ml was measured in one rabbit receiving the solution. This means that absorption of diazepam from the emulsion is immediate and thus, similar to the solution, sedative effect may be quickly achieved. Only very moderate prolongation of drug release can be obtained with the emulsion; the drug plasma levels in time period between 4 and 8 h were in the range 14.1–5.6 ng/ml and 7.7–2.9 ng/ml for emulsion and solution, respectively, and the difference was statistically significant ($P < 0.05$). The submicron emulsion as a vehicle does not alter pharmacokinetics of diazepam very much when compared with the currently available product, but elimination of ethanol makes such formulation safer. Our previous study on ocular delivery of pilocarpine prodrugs demonstrated a potential of submicron emulsion vehicle to reduce topical irritation [9]. This property may be also important for rectal delivery, since irritation or damage of the rectal mucosa is often evoked by other vehicles [15,16].

Rectal absorption of diazepam is poor when the internal phase of the submicron vehicle consists of a solid lipid. Both C_{max} and AUC_{0-24} are smaller after application of SLN than observed after administration of the solution or submicron emulsion and the difference is statistically significant. The low relative bioavailability, 47% compared to the solution, indicates that release of diazepam from SLN is incomplete. From the evidence that C_{max} appears at the first sampling time, i.e. after 20 min, it may be concluded that some of the drug was on the surface of the particles and this fraction was

Table 1
Diazepam pharmacokinetic parameters (mean ± SD) after rectal administration of the investigated preparations

Parameter	Solution	Emulsion	SLN
C_{max} (ng/ml)	48.86 ± 24.05	49.47 ± 17.03	24.88 ± 10.38[a]
T_{max} (min)	20 ± 0	40 ± 20[a]	23 ± 8
AUC_{0-24} (ng h/ml)	133.98 ± 42.29	186.80 ± 59.80	62.86 ± 12.67[a]

[a] Statistical difference with respect to the solution ($P < 0.05$).

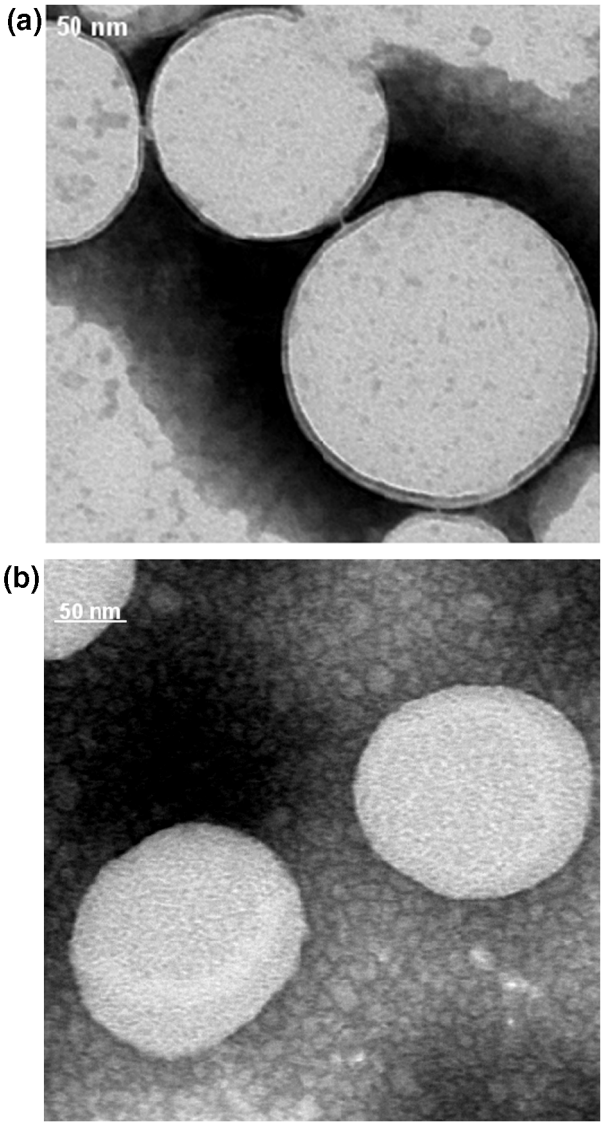

Fig. 3. Transmission electron micrographs of the SLN particles: (a) with diazepam; (b) unloaded.

immediately absorbed. This can be confirmed by transmission electron microscopy. Fig. 3 shows that a layer around the particles with diazepam is observed, which is not present in the unloaded SLN. This is also in agreement with other investigations, which postulate a drug-enriched shell around a core [12,17]. As already discussed above, we estimate that this fraction contains approximately 15% of the total diazepam dose. The other part of the drug is incorporated in a lamellar lattice structure of cetyl palmitate, as seen after structural investigation of the lipid [18]. Thus, the diffusion of the drug in the solid core is not efficient enough to allow a complete release. Lipid matrix, which is solid at body temperature is not advantageous system for diazepam rectal delivery, even if delivered as submicron dispersions. Therefore, the next experiments should be executed with lipids, which melt around a temperature of 38°C and so combine the fast drug release of the drug-enriched shell with the prolonged release of diazepam incorporated in the core.

The plasma concentration profiles presented in Fig. 2 show a second maximum at approximately 2 h in rabbits receiving solution and emulsion and at 1.5 h in those receiving SLN. The presence of the second maximum in diazepam plasma concentration after rectal delivery was also reported by Cloyd et al. [3]. Diazepam plasma concentrations determined in rabbits are much lower than those obtained in humans; 50–370 ng/ml plasma levels were obtained in humans after rectal administration of 5–10 mg of diazepam (0.07–0.7 mg/kg) [3,4]. This results from different pharmacokinetics [19,20]. Nevertheless for the purpose of comparison of different formulations the rabbit model can be accepted and similar conclusions may be expected also in humans.

4. Conclusions

Our results demonstrate that submicron emulsion may be a good choice of drug formulation when rectal delivery of diazepam is considered. It shows rapid and similar absorption as the solution. Absorption is only slightly sustained but elimination of organic solvents from the formulation makes it safer and more suitable as a pediatric drug. Replacement of the oily phase by lipids, which are solid at body temperature, results in reduced bioavailability of diazepam.

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