Improved Oral Delivery of Desmopressin via a Novel Vehicle: Mucoadhesive Submicron Emulsion[1]

Ehud Ilan,[2,3] Shimon Amselem,[2] Michael Weisspapir,[2] Joseph Schwarz,[2] Ayala Yogev,[2] Eduardo Zawoznik,[2] and Doron Friedman[2]

Received August 24, 1995; accepted March 30, 1996

Purpose. Desmopressin acetate (DDAVP) is used parenterally and intranasally in the control of several diseases. Oral administration of DDAVP, while most desirable, is not practical presently due to low bioavailability. The objective of the present study was to explore the feasibility for employing oil-in-water MucoAdhesive SubMicron Emulsion (MA-SME), a novel mucoadhesive vehicle with polymer-coated droplets, for enhanced oral delivery of DDAVP.

Methods. We used a modified pharmacopeal method, based on measurement of the antidiuretic activity, for the assessment of oral delivery of DDAVP in rats. DDAVP formulated in two MA-SME preparations, in non-mucoadhesive SME (plain-SME), in saline and in other control solutions was administered orally to rats via a stomach tube at a dose of 0.5 units/kg. At various times following DDAVP administration, water was given via a stomach tube. Excretion times for 30% and 60% of the total water load were measured.

Results. Excretion times for DDAVP in MA-SME formulations were always longer (up to 2-fold) than those following DDAVP in saline. By contrast, excretion times for DDAVP in plain-SME and in non-SME Carbopol (a Mucoadhesive polymer) solution were virtually identical to those for DDAVP in saline.

Conclusions. Formulations of MA-SME were shown to generate substantial enhancement (up to 12-fold) of the rat oral bioavailability of DDAVP with regard to simple saline solution of the drug. From the results it is also evident that MA-SME, but not plain-SME or non-SME Carbopol solution, is responsible for the enhancement of oral delivery of DDAVP in rats.

KEY WORDS: desmopressin acetate (DDAVP); peptide oral delivery; SubMicron Emulsion (SME); bioadhesion; mucoadhesion; mucoadhesive polymers.

INTRODUCTION

The interest in peptides as potent drugs increased dramatically in the last two decades due to recent advances in synthethic and molecular-biology techniques, enabling large-scale manufacture of these substances (1,2). However, with very few exceptions of small and cyclic peptides such as cyclosporin, most peptide drugs have low oral bioavailabilities with typical values of less than 1% (3). This phenomenon results mainly from the poor permeability of the intestinal mucosa to high molecular-weight peptides; the extensive proteolytic degradation of most peptides, both high and low molecular-weight by digestive enzymes in the gastrointestinal (GI) tract; the insufficient closeness of the drug or delivery system to the absorbing intestinal mucosa and its short residence time at the GI absorption site (3–5). Consequently, most new peptide-based drugs are administered via the parenteral route, a route which is not well accepted by patients, particularly for chronic therapy (4).

Still, for obvious reasons, the most desirable route is oral. The challenge here is to improve the oral bioavailability from less than 1% (the current value for nearly all peptide-based drugs) to at least 10%.

The goal of the present study was to investigate the feasibility for enhancement of the oral bioavailability of low molecular-weight therapeutic peptides by providing them proximity to the absorbing intestinal membrane via employment of a newly-developed MucoAdhesive Drug Delivery System (MA-DDS). The concept of augmentation of the oral delivery of drugs by utilization of MA-DDS is already known in the literature (6) and is based on strong attraction of the drug-containing MA-DDS to the mucus surface of the GI tract. This attraction is achieved by firm interaction between a special MucoAdhesive (MA) polymer, structurally integrated into the surface of the DDS, and the large glycoprotein molecules, named mucin, constituting the mucus lining of the GI surface epithelium (6).

Oil-in-water MucoAdhesive SubMicron Emulsion (MA-SME), a novel MA-DDS (see Fig. 1) the characterization of which is given elsewhere (7), was used in the present study as the DDS of choice and Desmopressin acetate (DDAVP) as the model peptide drug. DDAVP is a synthetic low molecular-weight polypeptide (1.18 kDa) structurally related to the posterior pituitary hormone arginine-vasopressin (antidiuretic hormone). This peptide is used parenterally and intranasally in the control of hemophilia A, von Willebrand disease, hemorrhage, nocturnal enuresis and diabetes insipidus (8–12). Following oral administration of DDAVP in human volunteers considerable portion of the drug is destroyed in the GI tract (13). Merely 0.7–1.0% of a given 100 or 200 μg dose appeared in the blood indicating that the bioavailability of this synthetic peptide, although somewhat higher than those of other small therapeutic peptides, is still either equal to or less than 1% (13,14) rendering it impractical for simple oral administration.

MATERIALS AND METHODS

Materials

Medium chain, capric/caprylic, triglyceride oil (Miglyol 812) was purchased from Huls, Germany; purified egg phosphatidylcholine (Lipoid E-80) from Lipoid, Germany. Tween-80 (Emulgin SMO-20) was obtained from Henkel, Germany. EDTA disodium dihydrate (USP) and glycerol (anhydrous, extra pure, USP) were from Merck, Germany. DDAVP (monoacetate trihydrate) was a gift from Mallinckrodt, USA. Carbopol 940 was purchased from B.F. Goodrich Co., Cleveland, Ohio and Methocel K4M from Dow Chemical Company, Midland, Michigan. α-Tocopherol acid succinate was obtained from Sigma Chemical Co., St. Louis, Mo., USA.

Preparation of DDAVP Formulations

Two different MA-SME formulations were prepared, one containing 0.05% Carbopol 940 (a crosslinked acrylic acid MA

[1] AAPS Ninth Annual Meeting, November 1994, San Diego, California, (Abstract No. PDD 7536).

[2] Pharmos Ltd., Kiryat Weizmann, Rehovot 76326, Israel.

[3] To whom correspondence should be addressed.

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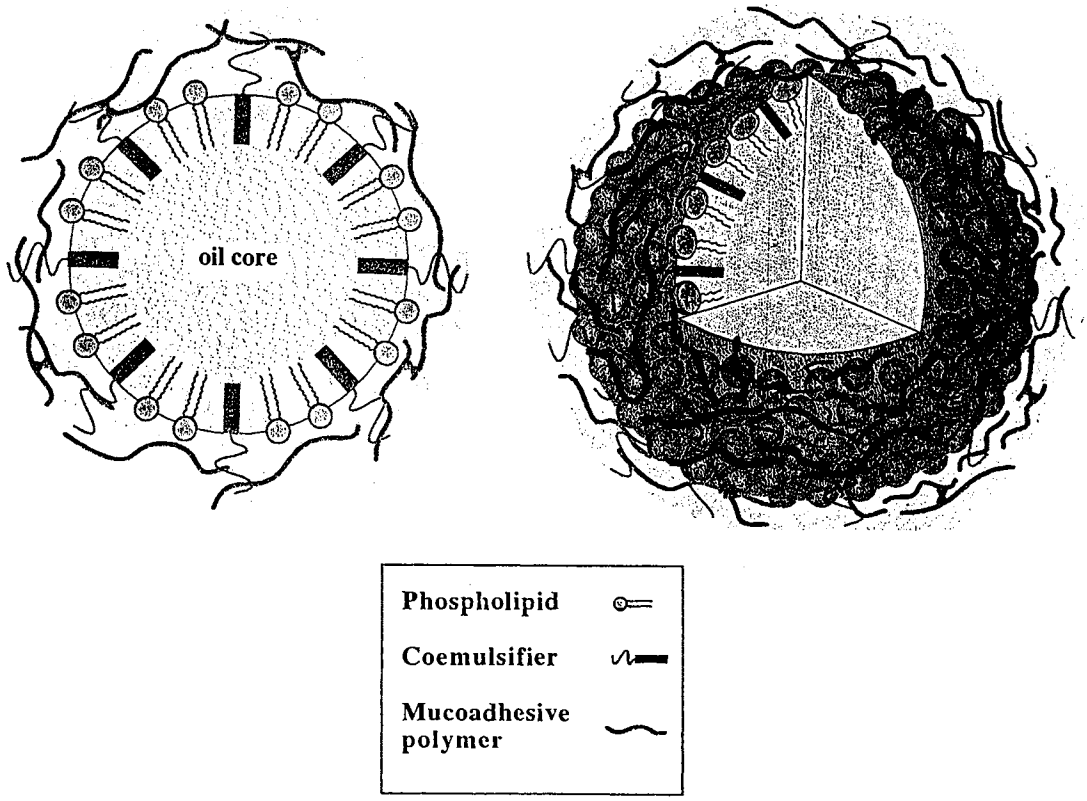

Fig. 1. Schematic structure for oil-in-water MucoAdhesive SubMicron Emulsion (MA-SME) droplet.

polymer) and the other containing 0.2% Methocel K4M (an hydroxypropyl methylcellulose (HPMC) MA polymer).

For 70 g batches of emulsions, the oil phases (5%, w/w) were prepared from 2.98 g Miglyol 812, 0.53 g Lipoid E-80 and 7.5 mg α-tocopherol acid succinate. All components were mixed by magnetic stirrer for 1 hr at 40°C. The aqueous phases contained 0.7 g of Tween 80, 0.175 g of di sodium EDTA, 70 μg of DDAVP (70 μl of 1 mg/ml solution of DDAVP in water) and were either 0.05% in Carbopol 940 or 0.2% in Methocel K4M. The resulting pH values of the aqueous phases were 4.5 ± 0.5. Aqueous phases containing 0.05% Carbopol were titrated to pH 7.5 in order to achieve essentially complete ionization of the carboxylic acid groups. The oil and aqueous phases were mixed by magnetic stirrer for 10 min, after which the coarse emulsion was dispersed by high pressure homogenization (Micron Lab 70, APV Gaulin, Germany) at 700 bar, 6 cycles, at a temperature lower than 40°C. Osmolality was brought to 300 ± 30 mOsm with glycerol and the final pH was adjusted to 4.5. The preparation was filtered through 0.45-μm PTFE membrane. The mean oil-droplet size was 100 ± 25 nm as measured with a Coulter N4MD particle-size analyzer (Coulter Electronics, England). A control non-mucoadhesive SME (plain-SME) formulation was prepared in the same manner but without a MA polymer. Plain (non-SME) solutions of DDAVP in 0.05% Carbopol 940 and in pure saline were also prepared as controls.

Biological Assay for Oral Delivery of DDAVP

Basic Oral Assay

Measurement of the antidiuretic activity of DDAVP administered subcutaneously into rats is a standard pharmacopeal method for the assessment of its biological potency (15). We used a similar modified method for the assessment of oral delivery of DDAVP in rats. The oral procedure was performed on Sprague-Dawley male rats weighing between 140 and 280 g, after overnight fasting. The range of weights in any one test was kept as small as possible and, in any case, did not exceed 50 g. Special metabolic cages (Model 3700MO-000, Tecniplast Co., Italy) were utilized for accurate collection of urine.

Optimization of the Administration Time

DDAVP formulated in MA-SME (0.05% Carbopol) or in saline was administered orally to rats via a stomach tube at a dose of 0.5 units/kg. At various times following DDAVP administration, water was given via a stomach tube. A first water load was equivalent to 5% and a second load (given 30 min later) to 3% of the animal's weight. Excretion time for 30% of the total water load was measured. Control experiments were done using the same water loads procedure but, with the omission of DDAVP.

Optimization of the Given Dose

Employing the optimal administration time found earlier, excretion time for 30% of the total water load was measured as a function of the orally administered DDAVP dose for DDAVP formulated both in MA-SME (0.05% Carbopol) and in saline. Again, the same procedure was used for control experiments except for the omission of DDAVP.

Calibration Curve for Evaluation of the Enhancement of Oral Bioavailability of DDAVP by Various SME Formulations

In order to be able to quantitatively evaluate the enhancement of the oral bioavailability for several SME DDAVP formulations with respect to simple saline solutions of this drug, the antidiuretic activities for saline solutions with various DDAVP concentrations were measured utilizing the aforementioned rat model. Employing administration times of 0.5 hr before the first water load the results for 30% excretion of the total water load are given in the calibration curve presented as Fig. 2. By entering the antidiuretic activity (expressed as excretion time in min) obtained with a specific SME DDAVP formulation possessing a known administered dose (Y units/kg) into the calibration curve, one can determine the DDAVP administered dose (X units/kg) that will provide identical antiduretic effect when formulated in saline solution. Division of X by Y will therefore give the enhancement factor of the oral bioavailability of this specific SME-formulated DDAVP with respect to simple saline solution of the drug.

RESULTS

Optimization of the Basic Oral Assay

Rat excretion time as a function of the administration time, for DDAVP formulated in MA-SME or in saline and given at a dose of 0.5 units/kg, is portrayed in Fig. 3. From the figure it is evident that the maximal antidiuretic activity for DDAVP in MA-SME is attained for administration time of 0.5 hr before the first load of water. This activity is about twice the activity of DDAVP in saline, showing maximal activity of orally absorbed DDAVP at this administration time.

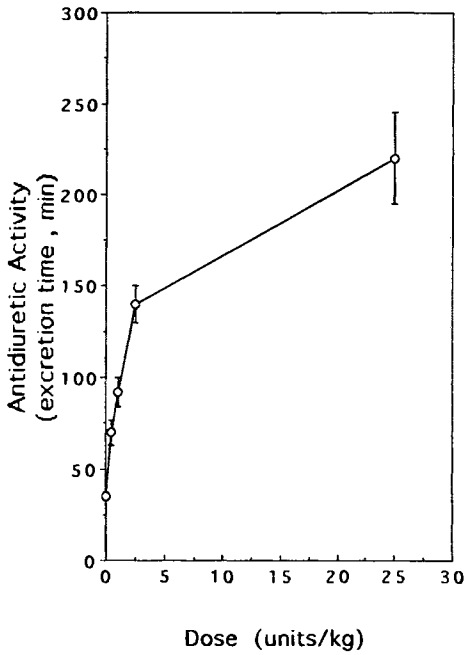

Fig. 2. Calibration curve for evaluation of the enhancement of oral bioavailability of DDAVP by various SME formulations. The dependence of the antidiuretic activity on the administered drug dose is given for saline solutions of DDAVP. Using administration times of 0.5 hr before the first water load the antidiuretic activity is expressed as the excretion time for 30% of the total water load.

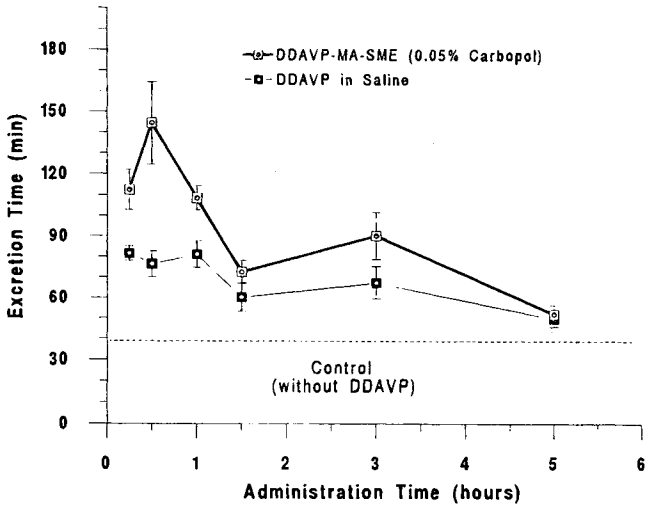

Fig. 3. Rat antidiuretic activity of DDAVP as a function of its administration time before the first water load. The antidiuretic activity is expressed as the excretion time for 30% of the total water load when DDAVP, formulated in MA-SME (0.05% Carbopol) or in saline, is administered at a dose of 0.5 units per kg rat body-weight. Each point represents the mean ± SE of four experiments.

Figure 4 shows a dose response curve for the antidiuretic activity of DDAVP in rats. The antidiuretic activity is expressed as the excretion time for 30% of the total water load when DDAVP, formulated in MA-SME (0.05% Carbopol) or in saline, is administered 0.5 hr before the first water load. The maximal difference between the antidiuretic activity for DDAVP in these two formulations is reached at a dose of 0.5 units/kg, displaying maximal potency of orally absorbed DDAVP at this dose.

Dependence of the Antidiuretic Activity of DDAVP on the Formulation Type

The influence of the DDAVP formulation type on the antidiuretic activity, at optimal dose and administration time

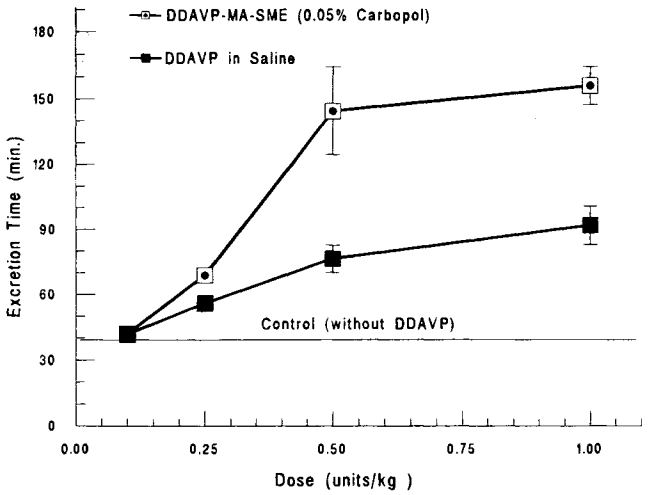

Fig. 4. Dose response curve for rat antidiuretic activity of DDAVP. The antiduretic activity is expressed as the excretion time for 30% of the total water load when DDAVP, formulated in MA-SME (0.05% Carbopol) or in saline, is administered 0.5 hr before the first water load.

conditions, is shown in Fig. 5 for various MA-SME, plain-SME and control preparations. Excretion times for 30% of the total water load are portrayed in Fig. 5a. From this figure one can see that the excretion time without DDAVP (the control) was 35 min. DDAVP in saline gave 70 min, very similar to DDAVP in non-SME 0.05% Carbopol Solution. DDAVP in MA-SME containing 0.05% Carbopol gave 156 min, demonstrating maximal antidiuretic activity for this formulation. DDAVP in MA-SME containing 0.2% HPMC shows some augmentation in the antidiuretic activity with excretion time of 102 min. Contrariwise, DDAVP in plain-SME shows virtually no enhancement in antidiuretic activity as compared with DDAVP in saline or DDAVP in non-SME 0.05% Carbopol solution. Very similar qualitative results are presented in Fig. 5b with respect to excretion times for 60% of the total water load.

DISCUSSION

Utilizing both the optimal DDAVP dose (0.50 units/kg) and the optimal administration time (0.5 hr before the first water load), the oral rat model elaborated here was used to compare the oral potency of DDAVP formulated in various MA-SME, plain-SME and control preparations.

The results portrayed in both Fig. 5a and 5b demonstrate considerable enhancement in the oral delivery of DDAVP for the 0.05% Carbopol-coated MA-SME formulation. DDAVP in MA-SME containing 0.2% HPMC shows some enhancement, while DDAVP in plain-SME shows virtually no improvement in the oral delivery of DDAVP as compared with DDAVP in saline. Quantitative evaluation of the enhancement of the oral bioavailability of DDAVP by the aforementioned two MA-SME formulations, can be judged by the calibration curve presented in Fig. 2. By entering the data obtained in Fig. 5a into Fig. 2, it is seen that the antidiuretic activity for 0.5 units/kg DDAVP in MA-SME containing 0.05% Carbopol (156 min) is equivalent to that obtained for about 6 units/kg of DDAVP formulated in saline, indicating that the oral bioavailability of DDAVP in this Carbopol-coated MA-SME formulation is roughly 6/0.5 = 12-fold increased with respect to saline solution of the free drug. On the other hand, the antidiuretic activity for 0.5 units/kg DDAVP in MA-SME containing 0.2% HPMC (102 min) is equivalent to that obtained for approximately 1.5 units/kg of DDAVP formulated in saline, pointing out that the oral bioavailability of DDAVP in this MA-SME formulation is roughly 1.5/0.5 = 3-fold increased with respect to saline solution of the drug.

From the results shown in Fig. 5 it is also evident that MA-SME is responsible for the substantial enhancement of oral delivery of DDAVP in rats, since neither non-SME Carbopol DDAVP solution nor plain-SME-DDAVP, when given orally, were able to increase the rat antidiuretic activity of DDAVP as compared to that produced by solution of the free drug. One probable explanation for this experimental conclusion is that a strong interaction between the mucin molecules, constituting the mucous lining of the GI mucosa, and the polymer coating

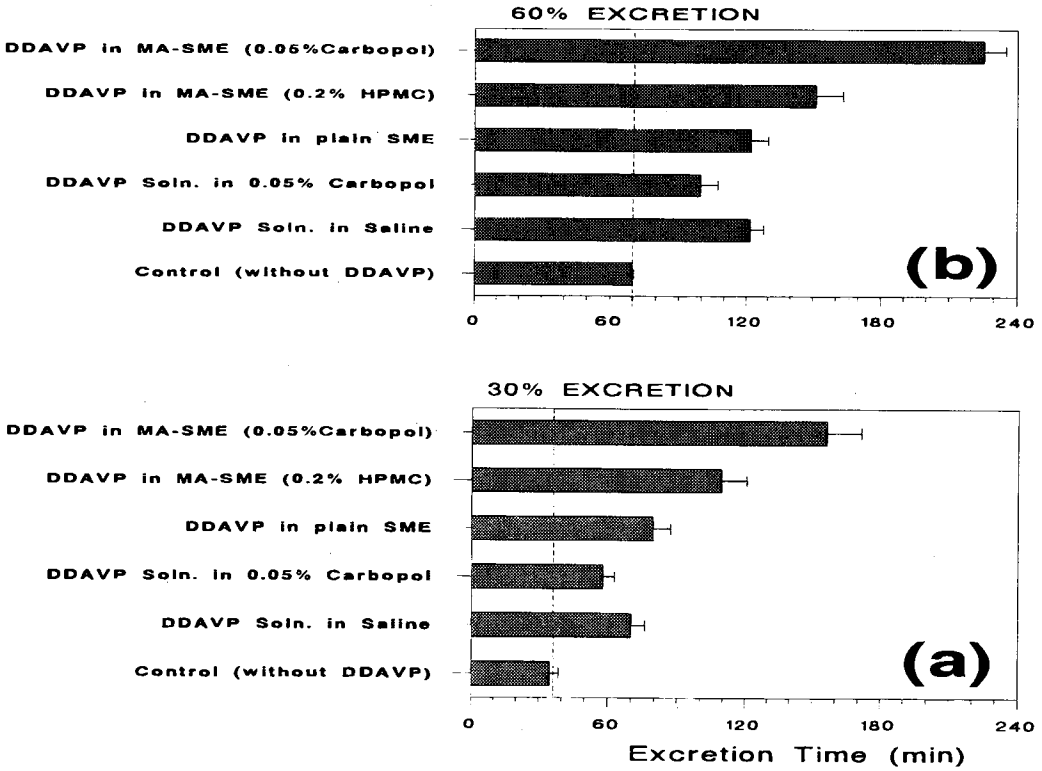

Fig. 5. Dependence of the antidiuretic activity of DDAVP on the formulation type. (a) The antidiuretic activity is expressed as the excretion time for 30% of the total water load when DDAVP, in various formulations, is administered 0.5 hr before the first load of water at a dose of 0.5 units per kg rat body-weight. (b) Same as (a) except that the antidiuretic activity is expressed as the excretion time for 60% of the total water load. Each point represents the mean + SE of 4 or 8 experiments for a and b respectively.

of the MA-SME promotes adsorption and prolongs retention of the emulsion particles in the intestinal mucosa. Since DDAVP is an hydrophilic molecule, considerable amount of the drug is assumed to accumulate at the oil-water interface, but as the formulation was adjusted to pH 4.5 where polyacrylic acid is not completely ionized a small fraction of the drug may also be dissolved in the oil phase. The adherence of the small submicron drug-carrier droplets to the mucous surface of the GI tract provides DDAVP with more opportunities to enter the circulation, thereby improving its oral delivery.

The ability of some mucoadhesive polymers, including Carbopol, to act as effective inhibitors of proteolytic enzymes was demonstrated recently by Luessen *et al.* (16). Assuming adherence of the drug-carrier droplets to the GI mucosa, there is, consequently, also a possibility that a Carbopol-facilitated protease-inhibition at the brush border is leading to the showed improvement in the bioavailability. According to this mechanism and in agreement with the experimental results, Carbopol non-SME solution will not cause noticeable improvement in the GI absorption of DDAVP. The reason for this is simple. The peptide molecule must be near the brush border and at the same site where the local Carbopol protease-inhibition takes place in order to be able to avoid the enzymatic barrier and this is only possible when the peptide is carried within or preferably at the oil-water interface of the SME particle.

Descriptions of possible mechanisms for penetration enhancement by polyacrylic acid were reported back in 1987 (17) and also recently (16). Thus, it is likewise feasible that local penetration enhancement, due to membrane alteration and augmentation of the epithelial permeability by Carbopol (a crosslinked acrylic acid polymer), is responsible for the improved GI absorption of DDAVP.

The results obtained in the present study with two MA polymers, Carbopol and HPMC, indicate that, in this case, the Carbopol was proved to be more MA than the HPMC. These findings are consistent with those reported by Smart *et al.* (18) for an *in vitro* assessment where the relative MA force (percentage of a standard) was 185 for Carbopol and 125 for HPMC, and those obtained by Chen and Cyr (19) for an *in vivo* evaluation. Thus, it seems that Carbopol being one of the most hydrophilic polyacrylic polymers is also one of the most MA polymers available in the market (6).

The Carbopol-coated MA-SME examined here—being made out of edible lipids and oils, egg lecithin and other biocompatible pharmaceutical-grade ingredients—was proved to be clinically safe (20). This DDS was shown, by the present study, as capable of increasing the oral bioavailability of DDAVP in rats 12-fold with respect to saline solution of the free drug. One wonders what is the actual oral human bioavailability of DDAVP formulated in Carbopol-coated MA-SME and whether or not this newly-developed DDS can serve for adequate oral human administration of the drug. Clarification of these issues await clinical investigation.

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