Thiolated Polymers: Development and Evaluation of Transdermal Delivery Systems for Progesterone

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Purpose. To evaluate the possible use of polycarbophil-cysteine (PCP-Cys) as polymeric matrix for transdermal progesterone application.

Methods. Thiolated polycarbophil was synthesised by the covalent attachment of cysteine to the basis polymer. The adhesive properties of PCP-Cys in comparison to polyvinylpyrrolidone/hydroxypropylmethylcellulose (PVP/HPMC) and polyvinylpyrrolidone/polyvinylalcohol (PVP/PVA) were investigated by testing the total work of adhesion (TWA) on porcine skin. Release studies in Franz diffusion cells and standard in vitro permeation experiments with porcine skin were performed analysing the progesterone content by high-performance liquid chromatography.

Results. Films based on PCP-Cys displayed very high cohesive properties due to the formation of interchain disulfide bonds. The TWA of the thiolated polymer on porcine skin was significantly ($P < 0.05$) the highest. In addition progesterone permeation was also the highest from PCP-Cys compared with PVP/HPMC and PVP/PVA within 24 hours.

Conclusion. PCP-Cys—a partly thiolated polymer—might be a novel polymer matrix for transdermal progesterone delivery with excellent adhesiveness on porcine skin.

KEY WORDS: progesterone; polycarbophil-cysteine conjugate; transdermal delivery; skin adhesion.

INTRODUCTION

Natural progesterone secreted by the corpus luteum exerts apart from its main progestational action (1) antiandrogenic activities (2,3), and also anti-mineralcorticoid effects (4,5). Until now, no synthetic progestin is able to mimic the same hormonal activities as the native hormone. However, if administered orally natural progesterone shows poor bioavailability due to its intense hepatic metabolism. This greatly limits the efficacy of peroral administration. Non-oral forms of progesterone have been conceived via the nasal, rectal, and vaginal routes. On the one hand, the nasal and rectal routes of application have not been found to be practical. On the other hand, there are only very few reports dealing with transdermal progesterone incorporated in ointments or gels. The disadvantage of such forms is the impractical use of a precise applicator for a defined amount of cream. Therefore, development of a patch delivery system similar to transdermal estrogen would have the benefit of a better compliance. Ultimately the success of such TDDSs depends on the ability of the drug to permeate skin in sufficient quantities to achieve its desired therapeutic effect. Among the various types, one uses the dispersion of a drug in an inert matrix made up of one or more polymers that release the drug at a controlled rate. The rate of drug release may be altered by variations of the dimensional parameters of the film, the polymer matrix material, and the drug concentration in the film. Recently published results from a study showed high permeation rates through excised rat skin from PVP/HPMC and PVA matrices containing urea and dexpanthenol, respectively (6). Because rat skin is known to be more permeable than human skin (7), the similar composed porcine skin was used (8). Proceeding on the presumption that PVP and PVA matrices are a good basis for progesterone, a mixture of both should also be tested.

Beyond it, reports of novel developed thiolated polymers, which have been designed for bioadhesive systems, give reasons for their use as matrices for transdermal delivery (9–11). One problem in developing transdermal patches is their insufficient adhesiveness on skin. In case of recently evaluated formulations of PVP/HPMC or PVA an additional adhesive has to be applied (6). In contrast, an excellent bioadhesiveness was measured for polycarbophil-cysteine conjugate (PCP-Cys) on porcine mucosa. Therefore, PCP-Cys also should be tested on whether a strong adhesiveness of this polymer system can be confirmed on skin. The aim of this study was to evaluate the possible use of PCP-Cys as a matrix for progesterone transdermal systems. For this purpose, the cohesiveness of the drug-polymer film and skin adhesion as well as release characteristics and permeation profiles of progesterone through porcine skin should be compared with PVP/HPMC and PVP/PVA matrices.

MATERIAL AND METHODS

Materials

Progesterone (Sigma, St.Louis, MO, USA); PVP according to Kollidon –30® (Basf, Ludwigshafen, Germany); PVA according to Mowiol 3-83 (Clariant, Frankfurt, Germany); and HPMC according to Metolose SH-4000 USP/NF. A copolymer of dimethylaminoethyl methacrylate and neutral methacrylic esters according to Plastoid E 35 L® (Röhm, Darmstadt, Germany) was used as adhesive. Triethylcitrate (Merck, Darmstadt, Germany) was used as plasticizer. The back foil was 3M/1109 Scotchpak, a polyester film laminate. The supporting foils were Scotchpak polyester film liners consisting of coated fluoropolymer, either type 3M/9743 or 3M/1022 (3M Medica, Borken, Germany). PCP (Noveon AA1, BF Goodrich, Becksville, OH, USA) was neutralized with NaOH as described previously (12). For the coupling reaction 1-ethyl-3-(3-dimethylaminopropyl) EDAC (Sigma, St. Louis, MO, USA) and L-cysteine hydrochloride monohydrate (Sigma-Aldrich, Steinheim, Germany) were used. All other chemicals used were of reagent grade.

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ABBREVIATIONS: TDDS, transdermal delivery drug system; TWA, total work of adhesion; MDF, maximal driving force; EDAC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; PCP, polycarbophil; NaPCP, polycarbophil neutralised with NaOH; PCP-Cys, polycarbophil-cysteine; PVP, polyvinylpyrrolidone; PVA, polyvinylalcohol; HPMC, hydroxypropylmethylcellulose; HPLC, high-performance liquid chromatography.

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Synthesis of PCP-Cys

One gram of the sodium salt of NaPCP was hydrated in 250 ml of demineralized water. The carboxylic acid moieties of the polymer were activated for 45 minutes with EDAC in a final concentration of 50 mM. Thereafter, 2 g of L-cysteine hydrochloride monohydrate was added to this reaction mixture. To gain different amounts of cysteine covalently attached to the polymer, the pH value was adjusted to the pH values listed in Table I by adding 5 M NaOH. The reaction mixture was then incubated for 3 hours at room temperature. The conjugate was isolated by dialyzing at 10°C in the dark against 1 mM HCl containing 2 μM EDTA, twice against the same medium but containing 1% NaCl and finally exhaustively against 0.5 mM HCl. The pH value of the dialysed polymer-cysteine conjugate was adjusted to pH 4.5 with 1 M NaOH. Thereafter the conjugate was lyophilized. A polymer being prepared and isolated in exactly the same way as the polycarbophil-cysteine conjugate but omitting EDAC during the coupling reaction served as control. The polymer-cysteine conjugate was stored at 4°C until further use. The amount of thiol groups are listed in Table I and the reaction scheme is presented in Fig. 1.

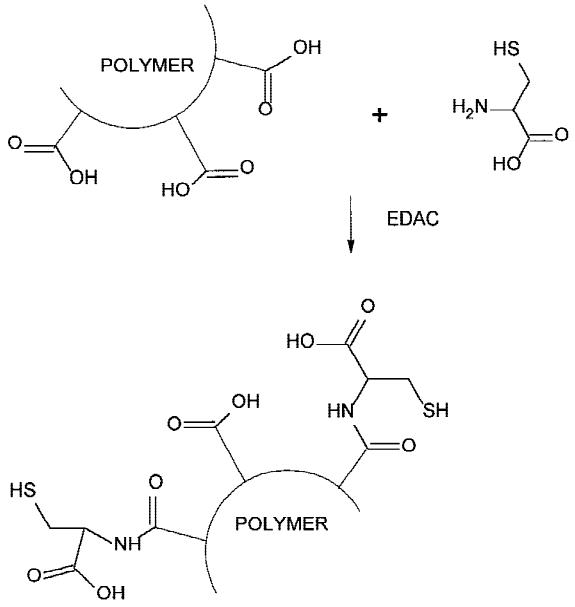

Fig. 1. Synthesis scheme for polycarbophil cysteine conjugates (PCP-Cys); EDAC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride.

Preparation of the TDDS

PVP/HPMC

First, 250 mg of progesterone were dissolved in 400 mg of ethanol (96 v%). Approximately 350 mg of PVP were dissolved in 1 ml of demineralized water and mixed with 650 mg of triethylcitrate and 6.25 g of aqueous HPMC gel (2%). To this mixture the ethanolic progesterone solution was added. After drying the polymer suspension at 40°C for 12 hours, the adhesive layer was applied by using a raquele and dried at 60°C for 30 minutes.

PVP/PVA

Initially, 300 mg of progesterone were dissolved in 400 mg of ethanol (96 v%). Five hundred milligrams of PVP and 1 g of PVA were dissolved in 5 g of demineralised water and 800 mg of triethylcitrate were mixed before the ethanolic progesterone solution was added. The polymer suspension was dried and the adhesive layer applied as described above.

PCP-Cys

First, 30 mg of PCP-Cys conjugates was swollen in 3 ml of demineralized water. Second, 125 mg of progesterone was dissolved in 250 mg of ethanol (96 v%). Thereafter, the progesterone solution was added to the swollen polymer. Then the polymer suspension was dried at 40°C for 12 hours.

Table I. Protocol for Synthesis of PCP-Cys Conjugates

Polymer	pH[a]	PCP (g)	Cyst (g)	EDAC (mM)	Thiol-groups (μMol/g)
PCP-Cys 1.5%	5	0.5	1	50	123.8
PCP-Cys 1.3%	4.5	0.5	1	50	107.3
PCP-Cys 0.8%	4	0.5	1	50	66.0

[a] pH during coupling reaction.

All resulting polymer/drug suspensions were continuously distributed by a raquele on a supporting foil fixed on a glass plate. Then the back foil was rolled on the top of the polymer or polymer-adhesive layer. For all experiments the matrix thickness was 250 μm.

Release/Permeation Studies From TDDS

The release of progesterone was investigated using Franz-type diffusion cells. The receptor compartment was filled with propylene glycol:water (40:60 w/w), themostated to 32°C and continuously stirred by a magnetic bar. Drug-polymer matrices came into direct contact with the receptor phase. The effective area available to diffusion was 1.13 cm^2. The receptor medium was replaced hourly by a fresh one.

Permeation profiles were determined using the same diffusion model but with porcine skin as native membrane. The excised skin was mounted on the cell, stratum corneum uppermost, with the dermal side facing the receptor compartment. The TDDS was placed on top of the stratum corneum. At defined time points the receptor medium was replaced. Samples from release as well as permeation experiments were analyzed by HPLC for progesterone content as described below. All experiments were repeated at least three times.

Analytical Procedure

Samples were assayed for progesterone content, using a previously reported modified HPLC method (13) at a flow rate of 1 ml/ min with a UV detector (series 200 LC, Perkin Elmer) at a detection wavelength of 240 nm. The stationary phase was a Nucleosil 100 5C-18 column (240 mm × 4.6 mm). Any polymer residues were held back on a pre-column (Nucleosil 100-5C-18, 40 mm × 4 mm). The mobile phase was methanol:water (90:10). Twenty-microliter samples were injected by an autosampler (ISS-200, Perkin Elmer). The retention time for progesterone was approximately 4.4 min. Cali-

bration curves were calculated on the basis of peak area measurements. The linearity interval established in the diffusion receptor phase of propylene glycol:water (40:60 w/w) was 0.01–54.5 μg/ml (r^2 = 0.999971) and in methanol 4.75–475 μg/ml (r^2 = 0.999900).

Determination of the Thiol Group Content

The amount of thiol groups on the polymer-cysteine conjugate was determined by iodometric microtitration (1 mN iod; indicator: starch) at pH 3 immediately after its synthesis and purification (values are listed in Table I) as well as from the final drug loaded film. Therefore, the polymer films were scratched off with a spatula and collected. Then a defined amount of demineralised water was added to cause swelling of the polymer. Afterwards, the pH of the swollen polymer system was measured. In case of the PCP-Cys (1.3 %), the pH of the swollen polymer was additionally adjusted to 6. These values are shown in Fig. 2 on the y-axes. Finally the thiol group content was analysed by microtitration after acidification with HCl to pH 3.

Drug Content

Samples of 5–10 cm^2 of TDDS were dissolved in 50 ml of methanol and sonicated. After filtering (minisart, Sartorius; 0.45 μm), the sample solutions were assayed by HPLC for progesterone content (Table II).

Tensile Studies

The adhesive strength of the polymers and drug containing films were evaluated with a peel adhesion test using a validated adhesive strength measuring device. A previously reported method for measuring mucoadhesion was modified for transdermal patches (11). Instead of porcine mucosa, abdominal porcine skin was used.

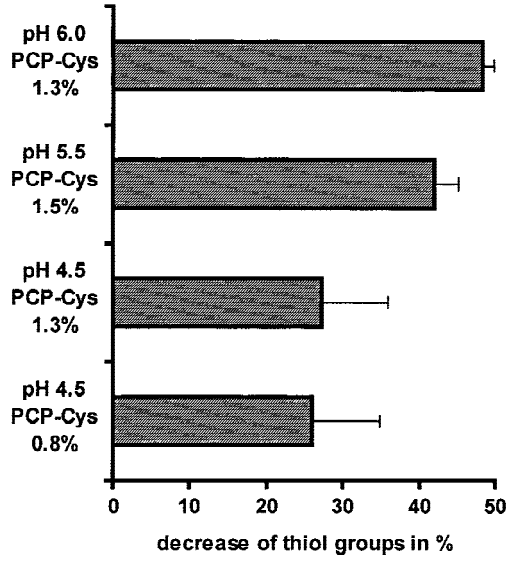

Fig. 2. Decrease of the thiol group content of the drug polymer film after drying in dependence of the pH.

Table II. Average Drug Loading per cm^2 of the Formulations

Formulation	Average progesterone content mg/cm^2
PVP/HPMC	1.45 ± 0.06[a]
PVP/PVA	0.81 ± 0.03
PCP-Cys	0.75 ± 0.10

[a] Mean ± S.D.

Experiments with Pure Polymer Mixtures Received by Direct Tableting

Eighty milligrams of the polymer mixtures PVP/HPMC, PVP/PVA, and PCP-Cys was compressed to flat faced discs (12-mm diameter; Hanseaten Exakta). The compaction pressure was constant during the preparation of all discs. The weight ratios of the polymers were according to the composition of the films.

Experiments with Drug Loaded Films According to the Formulations

In case of PVP/PVA, the dried film was blanked out in spherical discs (30 mm diameter). The discs were attached to the plug by a cyanoacrylate adhesive, in case of the two other formulations a defined amount of drug loaded film was poured on a plastic template of 30-mm diameter before drying.

In Fig. 3, the scheme of adhesive strength measuring device is shown. The porcine skin (**2**) was mounted on the top of a balance (**1**) (Mettler PC 4400) which was placed on a moving platform (**6**) and secured in place with a ring of lead. Then the tablet (**3**) or the patch (30 mm diameter) were attached with one side to a plug (**5**) using a cyanoacrylate adhesive. The plug is connected with a force transducer via a nylon thread (**4**). Then 100 μl of demineralized water were applied on about 10 cm^2 of skin. The platform was raised until the test disc attached to the porcine skin. After a contact time of 30 min the platform was lowered at a rate of 2 mm × min^{-1} until the test sample pulled clear off the membrane. The strength needed was registered and transferred to a PC for further

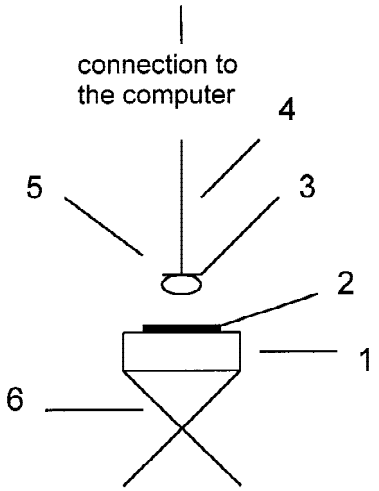

Fig. 3. Scheme of adhesive strength measuring device. Balance (**1**); excised porcine skin (**2**); tablet or patch (**3**); nylon thread (**4**); plug (**5**); moving platform (**6**).

processing. The total work of adhesion (TWA) representing the area under the force distance curve and the maximum detachment force (MDF) were determined using the WINLEDGE software (TAL Technology, Inc., Philadelphia) in combination with Excel 5.0 (Microsoft).

Data Analysis

Results are expressed as the mean of three to six experiments ± S.D. as indicated in the text. A *t* test with $P < 0.05$ as a minimal level of significance was used.

RESULTS AND DISCUSSION

Formation of Disulfide Bonds and Technical Properties

The synthesis of the conjugates was mediated by a carbodiimide reaction (Fig. 1). Cysteine was covalently bound to PCP by forming amide bonds between the primary amino group of the amino acid and the carboxylic moieties of the polymer. First evidence was the visual inspection of the polymer films. PVP/HPMC as well as PVP/PVA formulations showed homogenous films with suspended progesterone. As demonstrated in Fig. 4 (bottom) the cohesiveness of the pure PCP film was not sufficient to form a suitable homogenous polymer film and produced a fragile crackly layer. In contrast to this, inter- and/or intrachain disulfide bonds within the polymer itself are generated within PCP-Cys. This crosslinking is the reason for the excellent cohesiveness of the polymer films as seen in Fig. 4 top. The more thiol groups (Table I) are measured the higher can be the crosslinking process within the polymer and the better the technical properties of the films. This hypothesis could be confirmed by reduction of the disulfide bonds by the addition of dithiothreitol, which caused the same brittle and crackly films as pure PCP as in Fig. 4 (bottom panel). The thiol group content of the dry polymer, which was analyzed immediately after synthesis and purification versus the remaining thiol groups in the drug loaded film, is listed in Fig. 2. The formation of disulfide bonds can be proven indirectly by measuring the decrease of the thiol groups. If the polymer displays a pH value of above 5, the crosslinking process takes place to a high degree that is demonstrated by a high decrease of thiol groups. The crosslinking process of the polymer depends strongly on the pH value, for the same conjugate PCP-Cys (1.3% thiol group content) at pH 4.5 there is only a 1.3-fold decrease of thiol groups whereas at pH 6, a 1.9-fold decrease of thiol groups was detected. Results indicate the formation of disulfide bonds within the polymer, which are essential for the cohesiveness of the films. This assumption is confirmed by the visual inspection of the films. The more disulfide bonds are formed, the more cohesive are the resulting films.

Fig. 4. Top panel: PCP-Cys-drug film, bottom panel: PCP-drug film.

Tensile Studies

Transdermal patches are pharmaceutical sustained release devices that operate in a state firmly attached to human skin, therefore the adhesion properties are important attributes of this form of application. There are several theories of adhesion and underlying forces that appear to act independently (14,15). In tensile studies with porcine skin, the skin adhesion was measured from the pure polymer combinations of directly compacted discs as well as from the final drug containing compositions. The outermost layer of the skin is the stratum corneum, which consists of both lipophilic and hydrophilic domains as well as hair follicles and sweat glands. The hydrophilic parts consist mostly of keratin. Usually the stratum corneum has a water content of approximately 20%, which lies mainly in the keratin layers between the horny cells. The horny cells contain lipids in which the keratin filaments are dispersed. The elasticity of the skin itself is related to the water content and age. The surface energy values of dry clean skin reflects its dominant lipophilicity. The fact that more polar materials such as hydrogels may also adhere to skin may be in part due to the skin being wet and perhaps due to the presence of hair follicles and sweat glands which contain aqueous channels. In classic dermal systems pressure sensitive adhesives are used which have mainly lipophilic properties, here the novel approach is the application of a hydrophilic polymer system. Tensile studies performed with PCP-Cys, PVP/HPMC, and PVP/PVA demonstrated that PCP-Cys exhibits high adhesiveness compared to the other two polymer combinations (Fig. 5). The significantly highest total work of adhesion (TWA) was measured for PCP-Cys preparations the second highest for PVP/PVA and the lowest for PVP/HPMC formulations. Results for the final designed compositions with progesterone showed the same rang order but were in general lower. For the final design of the patch, it is important that the entire properties of the patch and the pharmacokinetic profile of the drug are investigated. The results of studies on the adhesive strength of the patch often can be related to the pharmacokinetic profile (16). The MDF was in good correlation with the total work of adhesion. Keratins represent the major components of the stratum corneum as well as of epidermal appendages as hair and nails (17). Keratins themselves are relatively poor of cystine but the keratin filaments are surrounded by an amorphous matrix of sulfur rich proteins. Disulfide exchange reactions between the sulfur containing polypeptide and the thiolated polymer are possible (9).

Release Studies

A comparison of the release rates from the three different formulations revealed no significant differences between the three formulations (Fig. 6). Although PVP/PVA and

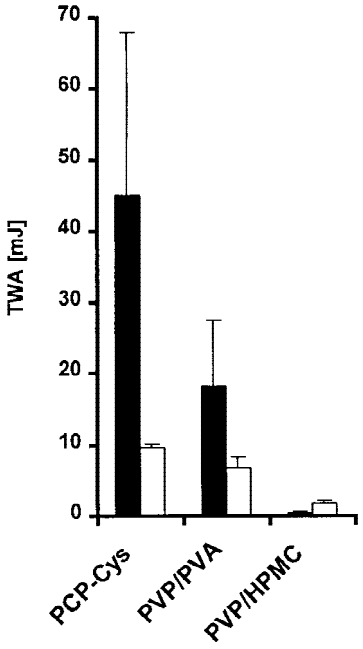

Fig. 5. Comparison of the adhesive properties of different polymer systems. Represented values are the means ± S.D. (n = 4–6) of the TWA (total work of adhesion) determined in tensile studies with compacted discs of indicated test material (black bars) and final designed drug loaded patches (blank bars).

PVP/HPMC contain an additional adhesive almost zero order release kinetics could thereby be observed. Whereas the dissolution rates from the formulations are similar high, the progesterone permeation through skin is significantly different.

Permeation Studies

The stratum corneum of pigs is similar to that of humans with respect to a considerable number of parameters and is therefore suitable for use in physicochemical and physical studies (18). Many similarities have been shown such as structure of collagen network, character of keratin proteins, composition of the lipid fraction of the skin surface and relatively high content of elastic fibers. Porcine and human skin gave comparable results in penetration studies with several drugs (8). Therefore, we used abdominal porcine skin unscalded for permeation studies.

In preliminary permeation experiments, two different conjugated PCP-Cys polymers were compared. After 24 hours, the progesterone permeation from PCP-Cys with 1.5% thiol group content was slightly decreased compared with the PCP-Cys with 0.8% thiol group content due to the higher crosslinking within the polymer with a higher thiol group content. Because of the coherent cohesive films, the applicability of the higher conjugated polymers was more favourable. Therefore, permeation comparisons were performed with PCP-Cys 1.5% (Fig. 7). The cumulative amount of progesterone permeated from PVP/HPMC and from PVP/PVA, respectively, was about 5.6 and 1.8 times lower within 24 h than that from PCP-Cys. One reason for the high permeation could be the high thermodynamic activity of such systems. For example, drug delivery rates for the lipophilic drug clonidin from hydrophilic polymers are higher than from hydrophobic ones and depend on the drug solubility (19). In contrast with PVP/PVA and PVP/HPMC-films the PCP-Cys-matrix showed an excellent cohesiveness over the whole period of time, therefore there is still a controlled permeation after 24 hours (Fig. 7).

Comment: Outlook

The major problem of progesterone is in bringing high amounts through the skin. It has been demonstrated *in vivo* that progesterone is absorbed through human skin from creams and luteal levels of serum progesterone can be achieved (20). A role of both the stratum corneum and the hair follicle has been proposed to influence the absorption rate (21). In a clinical study it has been investigated that a minimum offer of 30 mg of progesterone was necessary in order to get a measurable serum level. It has been proposed

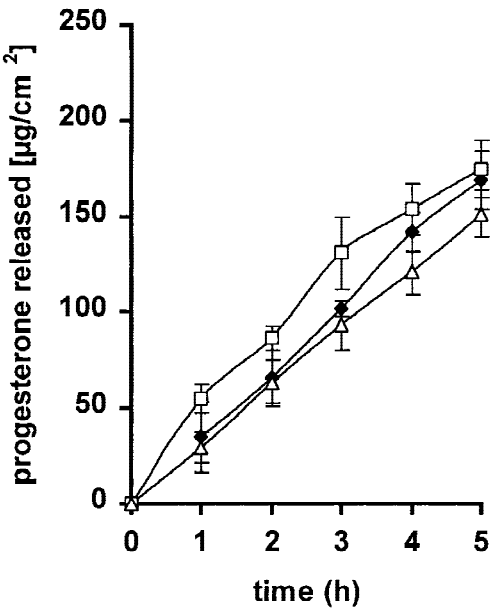

Fig. 6. Released progesterone from different TDDS formulations; ♦, PVP/PVA; △, PCP-Cys (1.5% thiol groups); □, PVP/HPMC; (n = 3).

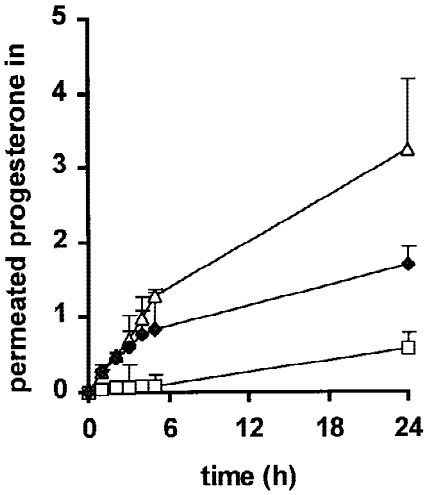

Fig. 7. Permeation of progesterone through porcine skin from different TDDS formulations. ♦, PVP/PVA; △, PCP-Cys (1.5% thiol groups); □, PVP/HPMC; (n = 3).

that 5 mg would clearly not be adequate to achieve therapeutic levels, because even the 30 mg of progesterone daily in 1 g of cream produced only a 1–2 ng/ml increase in some women. Burry et al. therefore conclude that 30 mg/day represent the lower end of the dosing range that should be considered in a long-term study. Therefore, it will be necessary to confirm in continued studies the optimum physiologic dose requirements and related frequency, duration and site of use of transdermal progesterone. For the TDDS, it would be easy to apply a delivery system with a diameter of 6 cm that could apply between 30 and 40 mg of progesterone and cause propable a measurable plasma concentration in the range of 1–2 ng/ml. A patch delivery system similar to transdermal estrogen would aid in ease of use and in high patient compliance. Thiolated polymers offer the possibility of high drug permeation and good adhesiveness on skin and might therefore be an interesting alternative to well established polymer systems.

CONCLUSIONS

PCP-Cys, a partly thiolated polymer is a novel matrix for transdermal delivery and exhibits the advantage of good cohesiveness within the polymer film and excellent adhesiveness on skin so that no additional adhesive is required. In studies with porcine skin a higher progesterone permeation could be demonstrated from PCP-Cys compared with PVP/HPMC and PVP/PVA formulations. Therefore, this thiolated polymer is a promising candidate as carrier for transdermal delivery systems.

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