Prodrugs of Gestodene for Matrix-Type Transdermal Drug Delivery Systems[1]

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Purpose. The aim of this study was to enhance the transdermal absorption of the highly active progestin gestodene from matrix type transdermal delivery systems (TDDS) by formation of prodrugs with improved matrix solubility.

Methods. Gestodene esters were synthesized via acylation of the drug with the respective carboxylic anhydrides. Subsequently TDDS were produced using the solvent cast method. Selected formulations were examined with in vitro diffusion experiments using skin of nude mice.

Results. One prodrug, gestodene caproate proved to be an oil at ambient temperature and showed a very high solubilty of over 10.5% in the TDDS matrix. Within in vitro penetration studies using those systems the prodrug exhibited a significantly higher transdermal penetration rate than gestodene from reference systems. Furthermore, the prodrug was hydrolyzed to the parent drug to a high extent during the passage of the skin.

Conclusions. Designing prodrugs to the requirements of matrix TDDS is an efficient way of enhancing the transdermal drug flux rate.

KEY WORDS: drug design; gestodene caproate; prodrug; progestin; transdermal drug delivery system.

INTRODUCTION

Transdermal application of female sex steroids has been an area of interest for the past 15 years (1). While in the beginning the research mainly focussed on the transdermal delivery of estrogens, especially estradiol, the transdermal application of progestins has also become an increasingly popular research target during the last decade. This has essentially been triggered by the protective effect which the progestins exhibit on the endometrium (2) within combined estrogen-progestin hormone replacement therapy. Furthermore, progestins offer the opportunity of transdermal fertility control due to their anti-ovulatory activity (3).

The present study deals with transdermal application of gestodene and several gestodene prodrugs. Especially the transdermal application of gestodene- and gestodene ester-containing matrix-type transdermal drug delivery systems (TDDS) was investigated. Gestodene (Fig. 1) is a highly active progestin which is currently used in combination with ethinyl estradiol in oral fertility control at dosages of 75 μg per day (4). In earlier studies, gestodene demonstrated fairly high steady state flux rates of approx. 0.6 $\mu g \cdot cm^{-2} \cdot h^{-1}$ after application to the back of postmenopausal women in form of prototypical topical solutions or suspensions, e.g. in 1,2-propanediol (5,6). Taking into account the high potency of gestodene as well as its substantial in vivo flux rates from prototype formulations gestodene was considered to be a promising drug candidate for further TDDS development. Matrix TDDS were the primary target of further investigations of transdermal gestodene delivery due to the attractiveness of their straightforeward construction type comprising only an impermeable backing, a matrix, and a release liner (7). Since the rate of percutaneous drug absorption follows to a certain extend the concentration of the drug within the matrix (8) especially systems with moderate to high drug loads are of interest. However, within early formulation work on gestodene it turned out that its solubility in standard polyacrylate adhesives is low and its incorporation into medical adhesives may lead to supersaturated systems. TDDS containing 2.2% gestodene e.g. showed a marked tendency of steroid recrystallization within a few months of storage (9), thereby revealing a too short shelf life. Previous attempts to solve the problem of physical instability of supersaturated gestodene TDDS focussed on the use of crystallization inhibitors and led to stable matrix TDDS containing a maximum load of 2% of the steroid (10,11).

The present approach was to design prodrugs of gestodene with significantly enhanced solubility in polyacrylate matrices. Especially a prodrug with a melting point below ambient temperature was aimed at, since the problem of drug crystallization during storage should be fully eliminated in this case.

MATERIALS AND METHODS

Synthesis

Gestodene (12,13) (drug grade, Schering AG, Berlin, Germany) was dissolved in pyridine and acylated using an excess of the respective carboxylic anhydride (synthesis grade, 99%) in the presence of 4-pyrrolidino-1-pyridine. After chromatographical purification on silicagel using hexane/ethyl acetate as eluent and/or crystallization the resulting esters were obtained in good yields (14). Elemental analyses were within ±0.4% of calculated values for C, H, O.

Gestodene Acetate

A 80.5 mmol reaction setup led to 25.11 g (88.5% yield) of gestodene acetate, m.p. 166.5°C (dichloro methane/diisopropyl ether), $[\alpha]_D^{22} = -128°$ (chloroform).

Gestodene Propionate

Yield 82.0%, m.p. 171.9°C (dichloro methane/diisopropyl ether), $[\alpha]_D^{22} = -117°$ (chloroform).

Gestodene Butyrate

Yield 90.0%, m.p. 150.6°C (hexane/ethyl acetate), $[\alpha]_D^{22} = -110°$ (chloroform).

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Gestodene Valerate

Yield 62.2%, m.p. 114.4°C (diethyl ether/diisopropyl ether), $[\alpha]_D^{22} = -102°$ (chloroform).

Gestodene Caproate

Yield 60.3%, oil, $[\alpha]_D^{22} = -101°$ (chloroform).

Calculation of Log P

was performed with the program C log P.

TDDS Production

The steroids (see Table II) were directely dissolved in polyacrylic pressure sensitive adhesive Gelva™ 788 (50% solids in ethyl acetate, Solutia, Springfield, MA, USA). Subsequently the wetmix was coated on siliconized polyester (Bertek Inc., St. Albans, VT, USA) using an Erichsen apparatus, model 335/I (Hemer-Sandwig, Germany). After 20 min of oven drying at 70°C lamination with Saran™/Hytrel™-coextrudate (Bertek, s.a.) was carried out. The resulting three-layered sheets had a coatweight of 56 to 71 g · m^{-2}. TDDS of different sizes were diecut from the sheets for further examination.

Polarized Microscopy

The search for drug crystals was performed using a Leitz Laborlux S with a linear polarization equipment (Leitz, Bensheim, Germany). TDDS of 10 cm^2 in size were removed from the release liner and applied onto an object slide.

HPLC Measurements

The HPLC equipment was operated using Access*Chrom software (Perkin Elmer, San Jose, CA, USA) for control and calculation purposes. It comprised a Waters WISP 712 (Milford, MA, USA) as sampler, a Brownlee Labs Inc. mixing chamber (BAI, Bensheim, Germany), and an ABI UV/VIS detector (Foster City, CA, USA) at 242 and 227 nm. A 125 × 4.6 mm steel column filled with Hypersil ODS 3 μm (Bischoff, Leonberg, Germany) was used as stationary phase and mixtures of acetonitrile and water (gradient from 35% to 65% water) served as an eluent. For the determination of drug content of the patches, the matrix weight was determined in the first place. Subsequently the patches were disintegrated by sonification in acetonitrile and 50 μl-aliquots of the resulting mixtures injected into the system described above. In order to determine the concentration of gestodene and its prodrugs in the fractions obtained during penetration studies (see below) aliquots of 50 μl of the receptor phase were injected without further dilution.

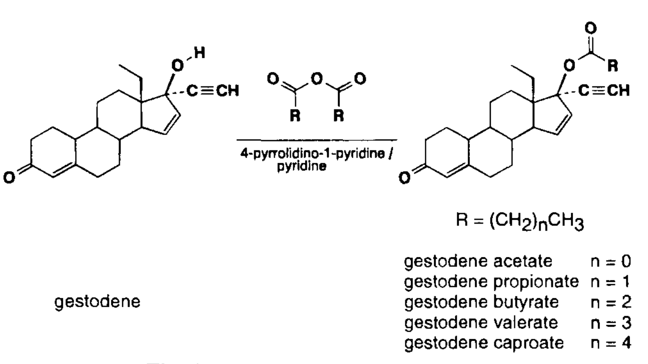

Fig. 1. Synthesis of gestodene esters.

Determination of Transdermal Flux Rates

The percutaneous steroid absorption was investigated following application of four types of TDDS containing either gestodene or one of its prodrugs gestodene valerate or gestodene caproate (ref. to Table II). The study was performed using a battery of 12 FRANZ-flow-through-diffusion-cells and full thickness skin of nude mice. Each TDDS was tested on 3 skin samples prepared from 3 different animals. Thus, the diffusion cell battery allowed simultaneous testing of the 4 preparations in triplicate. Male nude mice (strain: swiss nude; breeder: Schering AG, age: ca. 8–9 weeks) were sacrificed by diethylether narcosis. From each animal, 4 pieces of ca. 1–2 cm^2 ventral or dorsal skin were prepared immediately before mounting the skin onto the Franz-diffusion-cell. The subcutaneous fat was removed carefully. TDDS of 12 mm in diameter were applied on a skin surface of about 1 cm^2 while the actual diffusion area of the FRANZ-cell was 0.32 cm^2. The receptor fluid consisted of 40% PEG 400 in bidestilled water (v/v) supplemented with 1000 I.E. penicilline G (Hoechst AG, Frankfurt, Germany) per ml. PEG 400 was added to increase the solubility of the lipophilic steroids and thereby to provide sink conditions throughout the experiment. Penicilline G was added to inhibit growth of microorganisms. The flow-through volume of the FRANZ-cells was 0.5 ml receptor fluid per h. All fractions were frozen at latest 12 h after sampling and kept at −30°C until approximately 12 h before HPLC analysis.

The steroid concentration in the receptor fluid was monitored as a function of time up to ca. 54 h by means of the HPLC system described above. For gestodene caproate containing TDDS (TDDS-3 and TDDS-4, ref. to Table II) the total steroid flux could only be calculated up to 33 h due to overlapping of the gestodene peak with an interfering peak after 33 h. However, the concentration of gestodene caproate was measured up to 48 h.

Determination of Drug Release Rates

Drug release from the patches was determined over a 48 h time period by the paddle-over-disk method (USP XXIII), using 10 cm^2-patches, 500 ml of 40% PEG 400 (s. a.), and the HPLC method described above.

RESULTS

Gestodene esters were synthesized according to the reaction scheme given in Fig. 1. They were purified via column chromatography and obtained in good yields. The octanol water partition coefficient and the melting points of gestodene and its n-alkyl esters are given in Table I.

The lipophilicity of the compounds increased with acylation of the hydrophilic 17β-hydroxyl group of the steroid and with higher chainlength of the carboxylic acid residue. Thus, the log P value grew by one order of magnitude due to acetylation and additionally by half an order of magnitude for each methylene group inserted between carboxyl function and methyl residue.

Table I. Octanol-Water Partition Coefficient, Melting Points, and Polyacrylate Solubility of Gestodene and Its Prodrugs

Compound	Abbreviation	log P[a]	m.p. (°C)	Solubility in polyacrylate matrix
gestodene	GTD	2.46	200–202 (24)	≈1%
gestodene acetate	GTDA	3.36	166.5	<3%
gestodene propionate	GTDP	3.89	171.9	<2%
gestodene butyrate	GTDB	4.42	150.6	[b]
gestodene valerate	GTDV	4.95	114.4	<4%
gestodene caproate	GTDC	5.48	[c]	≥10.5%
gestodene oleate	GTDO	11.3	[d]	[d]

[a] Partition coefficient was calculated using the program C log P (25).
[b] Not tested.
[c] Oil at 4–8°C.
[d] Not available, since compound was not synthesized.

The melting points of the compounds generally decreased with higher chainlength of the pro-moiety, with the exception of the acetate which exhibits a m.p. 5°C lower than the propionate. Gestodene caproate however did not crystallize at all, even after thorough purification and storage at 4 to 8°C. It even exceeded the target criterion of being an oil at ambient temperature. Therefore, it could also not crystallize in TDDS matrices.

Gestodene, its acetate, propionate, and valerate ester were selected for initial formulation experiments. Microscopic search for drug crystals within the resulting matrix patches directly after manufacturing as well as after storage for up to 7 months at 4, 25 and 40°C revealed that the loading capacity of polyacrylate matrices is approximately 1% for gestodene, <3% for its acetate, <2% for its propionate, and <4% for its valerate (see Table I).

Gestodene valerate together with the oily caproate were selected for in vitro penetration studies. A second set of TDDS comprising the steroid, polyacrylate pressure sensitive adhesive, Saran™/Hytrel™ backing, and PET release liner was prepared within a discontinuous process. Subsequently, all patches were analyzed by HPLC for drug content and by polarization microscopy for absence of drug crystals (see Table II).

FRANZ-flow-through-diffusion-experiments were performed following application of the TDDS to full thickness skin of nude mice. The time curves of cumulatively absorbed progestin doses are represented in Fig. 2. For the sake of comparability all absorbed doses were corrected by molecular weight and are given as gestodene equivalents. The average percutaneous fluxes of parent drug and prodrugs are given in Fig. 3.

Table II. Drug Load of the TDDS Introduced into the *In Vitro* Penetration Study

TDDS type	Drug	Drug load (%)	Molal drug load vs. reference
TDDS-1 (= reference)	GTD	2	(64 mmol·kg^{-1})
TDDS-2	GTDV	5.1	double molal
TDDS-3	GTDC	2.6	equi-molal
TDDS-4	GTDC	10.5	four-fold molal

Note: All TDDS were checked for drug content by means of HPLC and proved to be within 88 to 101% of the target drug load; all patches proved to be free of drug crystals by microscopic examination.

Due to the fact that within the experiments with GTDC containing patches GTD flux determinations were only possible up to 33 h (refer to Materials and Methods section) the comparative discussion of all four formulations concentrates on the time frame of 0 to 33 h after application. During the 33 h time course of the skin penetration experiment no significant effects related to potential degradation of the skin were seen. E.g. the slopes of the cumulatively absorbed doses are nearly linear, which would not be the case if disintegration of the skin troughout the experiment took place. These findings in principle correspond to the findings of other researches.

Within 33 h after application of **TDDS-1** containing 2% GTD, a total of 3.13 ± 2.45 μg GTD (8.2 ± 6.4 % of the dose; all values are given as mean ± standard deviation) was cumulatively absorbed through the skin. After an average of about 10 h the percutaneous GTD concentrations exceeded the limit of quantification and the transdermal flux reached 0.03 $\mu g \cdot cm^{-2} \cdot h^{-1}$, while at 26 h the maximum percutaneous GTD flux of 0.51 ± 0.39 $\mu g \cdot cm^{-2} \cdot h^{-1}$ was observed. The mean percutaneous GTD flux was 0.30 ± 0.22 $\mu g \cdot cm^{-2} \cdot h^{-1}$ during the first 33 h of the experiment. **TDDS-2** containing 5.1% GTDV delivered 4.95 ± 0.73 μg GTD (6.5 ± 1.0% of

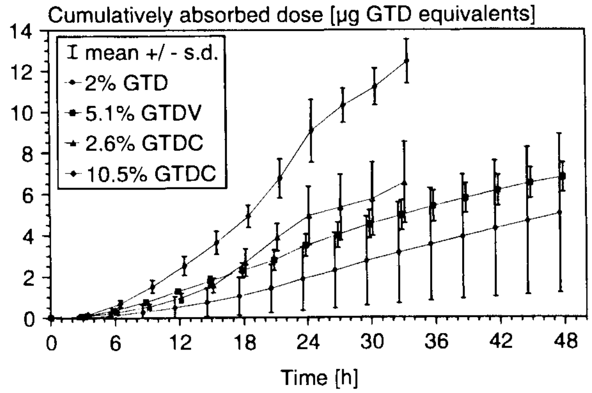

Fig. 2. Cumulatively absorbed progestin dose following dermal application of four matrix TDDS. Because GTDC was not completely hydrolyzed throughout the whole experiment, the skin penetration of GTD and its prodrugs was compared using the total progestin flux of GTD and the respective ester. Percutaneous fluxes were expressed in μg GTD equivalents $\cdot cm^{-2} \cdot h^{-1}$ taking into account the respective molecular weights.

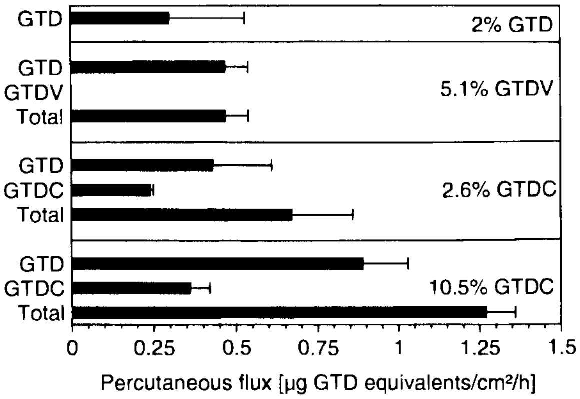

Fig. 3. Mean percutaneous fluxes of gestodene and its prodrugs from four different matrix TDDS.

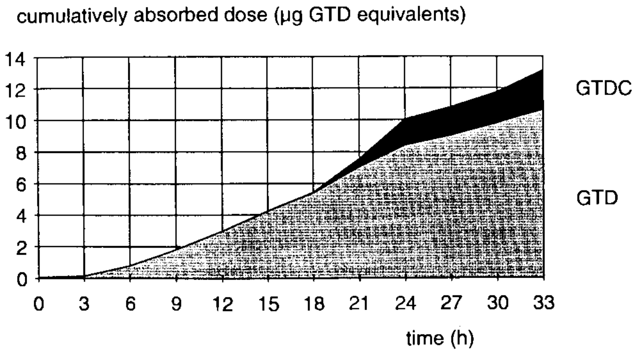

Fig. 4. Cumulative absorption of gestodene and gestodene caproate after application of a matrix TDDS containing 10.5% gestodene caproate (TDDS-4).

the dose) within 33 h of experiment. The GTD concentrations exceeded the limit of detection 3 h after application and maximum percutaneous GTD fluxes of 0.75 ± 0.06 µg · cm^{-2} · h^{-1} were observed at about 23 h. The mean percutaneous GTD flux was 0.47 ± 0.07 µg · cm^{-2} · h^{-1} and no GTDV was detected in the receptor fluid throughout the whole experiment, thereby indicating complete hydrolysis of the ester bond during skin passage.

From **TDDS-3** containing 2.6% GTDC 6.49 ± 1.98 µg gestodene equivalents (16.9 ± 5.2% of the dose) were cumulatively absorbed through the skin. After less than 3 h upon application, the GTD concentration exceeded the limit of detection and at 20 h a maximum percutaneous GTD flux of 0.83 ± 0.30 µg · cm^{-2} · h^{-1} was observed. The mean percutaneous GTD flux was 0.43 ± 0.18 µg · cm^{-2} · h^{-1} and within the first 12 h p. a. no GTDC was detected in the receptor fluid. Later on, also non-hydrolyzed GTDC penetrated the skin indicating that the metabolic activity of the skin esterases decreased in the course of the in vitro experiment. The maximum GTDC flux of 0.71 ± 0.16 µg GTD equivalents · cm^{-2} · h^{-1} was observed 20 h after application. Mean and maximum total progestin fluxes were 0.61 ± 0.19 µg GTD equivalents · cm^{-2} · h^{-1} and 1.46 ± 0.27 µg GTD equivalents · cm^{-2} · h^{-1}, respectively. Transdermal gestodene delivery was highest with **TDDS-4**, containing a drug load of 10.5 % GTDC. Within 33 h of experiment 12.38 ± 1.09 µg GTD equivalents (8.1 ± 0.7% of the dose) were cumulatively absorbed. Less than 3 h following application, the GTD concentration exceeded the limit of detection and a maximum percutaneous GTD flux of 1.49 ± 0.21 µg · cm^{-2} · h^{-1} was observed at 19 h. The mean percutaneous GTD flux was 0.89 ± 0.14 µg · cm^{-2} · h^{-1}. Within the first 18 h p. a., no GTDC was detected in the receptor fluid. Later on, also unchanged GTDC was detected in the receptor fluid (see Fig. 4).

A maximum GTDC flux of 1.39 ± 0.19 µg GTD equivalents · cm^{-2} · h^{-1} was observed 24 h following application. Respective mean and maximum total progestin fluxes were 1.17 ± 0.10 µg GTD equivalents · cm^{-2} · h^{-1} and 2.54 ± 0.41 µg GTD equivalents · cm^{-2} · h^{-1}.

In order to clarify the impact of the synthetic variations of gestodene onto drug release rates from matrix TDDS paddle-over-disk experiments (USP XXIII) were run over a 48 h time period. Fig. 5 shows the release profiles for gestodene (TDDS-1), gestodene valerate (TDDS-2) and gestodene caproate (2.6% = TDDS-3; 10.5% = TDDS-4) from four matrix TDDS. The cumulatively absorbed progestin doses (CAPD) derived from the skin permeation experiment after 33 h (see also Fig. 2) were included into this representation in order to enable a direct comparison of drug release and in vitro absorption.

Figure 5 shows that the fastet relative drug release was obtained from the gestodene containing reference TDDS, which exhibited 95.2% drug release already after 24 h. Relative drug relase from the gestodene valerate containing TDDS-2 was slower (72% after 24 h) and slowest from the two gestodene caproate containing patches. After 24 h 42.8% of the compound was released from TDDS-3, and 37.9% from TDDS-4.

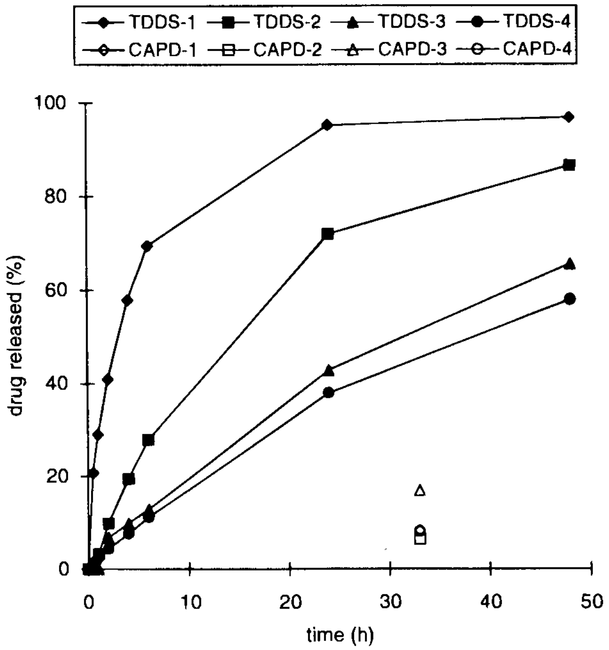

Fig. 5. Release (closed symbols) of gestodene and its prodrugs from four different matrix TDDS. The cumulatively absorbed progestin dose (CAPD, open symbols) from each formulation is included at the time-point 33 h for the purpose of direct comparison of drug release and drug absorption.

DISCUSSION

It is known from the literature that transdermal delivery of drugs can be improved by various mechanisms, e.g the use of skin penetration enhancers (15) and the increase of the drug load of the system (8). In the past, several studies were successfully carried out to identify penetration enhancers for GTD (5) and also the concentration of gestodene could be raised to 2% in supersaturated matrix-type TDDS stabilized by the addition of crystallization inhibitors (10,11).

A further approach to enhance transdermal drug penetration is the optimization of the drug itself via formation of prodrugs. While formation of prodrugs for transdermal application has been an area of interest for many years (16), little attention has been drawn to tailor made compounds for matrix TDDS so far. However, especially matrix TDDS based on pressure sensitive polyacrylate adhesives have become increasingly popular. This is mainly due to their high wearing comfort resulting in good compliance.

Since the main problem of gestodene-containing matrix TDDS was the recrystallization tendency of the drug at higher loading levels, the objective of the present study was to design a prodrug which should not crystallize during storage of the TDDS, preferably a compound which was an oil at ambient temperature. The work focussed on the synthesis of esters of carboxylic acids with gestodene since these promoieties exhibit very low toxicity, due to the convenient access to esters of 17-hydroxy steroids, and due to their known susceptibility to skin esterases (17). The first idea was to acylate gestodene with activated oleic acid which may have led to an oily compound due to the bulkiness and Z-configuration of the acyl residue. However, this idea was withdrawn since the calculation of the octanol water partition coefficient of gestodene oleate led to a log P value of 11.3. Any compound bearing such a high log P would most likely be much too lipophilic to enter the viable epidermis and therefore may show low transdermal flux rates. Since it is known from several homologous series of esters of steroids that also shorter side chains may lead to oily compounds (18,19) the final concept was to increase the length of the side chain of gestodene esters methylene group by methylene group, until the first oily compound was obtained. Thus the acetate, propionate, butyrate, valerate, and caproate esters of gestodene were synthesized. The melting points of all prodrugs were lower than the melting point of the parent drug and with gestodene caproate an oily compound was obtained. All prodrugs exhibited a higher solubility than gestodene in polyacrylate matrices, and the solubility of the compounds increased with decreasing melting points. It could be raised from approximately 1% for gestodene to ≥10.5% for gestodene caproate which is an increase by more than a factor of 8 on a molal basis. This is also a significant higher drug load compared to matrix TDDS containing 2% gestodene stabilized by the addition of crystallization inhibitors (11). Hence, prodrug formation was a success from the point of view of stabilization of highly loaded TDDS against drug recrystallization.

However, the question remained to be answered whether the extremely high drug load of 10.5% would also lead to high drug fluxes. Therefore, GTDC and in addition the relatively low melting GTDV were selected for in vitro penetration studies in comparison to gestodene itself. Nude mouse skin as was used as a model due to its good accessability, its common use within transdermal in vitro studies (20), and its known metabolic activity (21). Although the permeation rate of a compound through nude mouse skin is not predictive for human skin in its absolute values, this model proved to be useful to obtain a relative judgement when comparing different drugs (5,22). In the present study it turned out that the highly loaded TDDS-4 (containing 10.5% GTDC) provoked significantely higher mean progestin fluxes than TDDS-1 (2% GTD), TDDS-3 (5.1% GTDV), and TDDS-4 (2.6% GTDC). Comparison of the cumulatively absorbed progestin doses (CAPD, 33 h time point included into Fig. 5) with the drug release data of the patches revealed that the latter is higher than the former for all four matrix-type TDDS. Hence, prodrug formation did not result in compounds with too poor release rates in this case and the release is not the rate limiting step within the entire process of transdermal drug absorption. Therefore, the rate limiting step is allocated to the skin. To further clarify whether e.g. the permeation of the stratum corneum or of the epidemis was the rate limiting step of transdermal absorption of the new prodrugs was not within the scope of this study.

The results of the present study indicate, that the esterases present in skin of nude mice are able to hydrolyze both gestodene prodrugs. Gestodene valerate, whose fluxrate was as high as the fluxrate of gestodene caproate after application of TDDS-3, was fully hydrolyzed throughout the whole experiment. No indication of any potential enzyme saturation could be derived from the present data for the valerate. Since the steady state flux of the compound was reached already after approximately 10 h it also seems to be unlikely that extension of the experiment beyond 48 h would have allowed to identify a timepoint of potential ester saturation.

Within the first 12–18 h of the experiment also the homologous caproate ester was completely hydrolyzed from both test formulations TDDS-3 and TDDS-4, but later on also unchanged GTDC was detected in the receptor phase. However, the occurrence of GTDC in the acceptor medium does not indicate saturation of the skin esterases. If saturation of the skin esterases would have taken place the hydrolysis rate of GTDC should be significantly lower after application of TDDS-4 than after application of TDDS-3, since the total progestin flux was significantly lower in the latter case. On the other hand there is a time dependent decrease of gestodene caproate hydrolysis after application of TDDS-3 as well as TDDS-4. This decrease is most likely caused by reduction of skin viability over time within our in vitro model, which does not comprise any salts and nutritians in the acceptor medium.

The current data showing that the hydrolysis rate is lower for the somewhat more bulky caproate ester than for the valerate correspond well to findings with other steroid esters (23).

Finally, on the basis of the presented results it is concluded that the low melting gestodene caproate optionally combined with a suitable estrogen may be a useful prodrug for the development of attractive matrix-type TDDS for hormone replacement therapy as well as transdermal fertility control.

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