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ORIGINAL ARTICLE

Development of microemulsion of mitotane for improvement of oral bioavailability

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Abstract

Background: Mitotane (*o,p'*-DDD) is considered to be the drug of choice in the treatment of nonresectable and metastasized adrenocortical carcinoma. However, mitotane has poor solubility in the gastrointestinal tract and very low bioavailability. Consequently, to achieve therapeutic plasma level, high cumulative doses (4–6 g/day) of mitotane were usually used during 3–5 months. To shorten this equilibration time and reduce gastrointestinal side effects, a self-microemulsifying drug delivery system (SMEDDS) of mitotane has been developed. *Method*: First time, the solubility of mitotane was determined in various oils and surfactants; then, the influence of oils, surfactants, and cosurfactants on the formation of SMEDDS was investigated by constructing ternary phase diagrams. SMEDDS was characterized by morphological observations and droplet size measurements. Intestinal drug permeation of SMEDDS of mitotane (3 mM) was assessed in an Ussing-type apparatus and the bioavailability was determined in a rabbit model. *Results*: The optimum formulation consisted of a mixture of Capryol®, Tween®, and Cremophor® EL (33:33:33). The formulation was found to pass through the intestinal barrier much faster than a solution of mitotane (14.85 ± 0.8 versus 3.03 ± 0.2 µmol/cm^2). Moreover, after oral administration in rabbits, the relative bioavailability was 3.4, compared with that of the conventional form (Lysodren®). *Conclusion*: This SMEDDS can now be considered as a very good candidate to optimize the administration of mitotane.

Key words: *Bioavailability; microemulsion; mitotane; oral route; pharmacokinetics*

Introduction

Mitotane (*o,p'*-DDD, Figure 1), an adrenolytic agent, is considered to be the drug of choice in the treatment of nonresectable and metastasized adrenocortical carcinoma[1]. A 30% objective response rate has been observed in patients with adrenocortical carcinoma, but cure is rarely achieved and its impact on survival is questioned[2]. This response rate could increase to 55–66% if plasma mitotane levels reach 14 mg/L[3,4]. Additively, its use has been shown to be beneficial as an adjuvant treatment, after surgery[5].

However, mitotane has poor solubility in the gastrointestinal tract. After administration of 10 g/day (capsules of mitotane), the bioavailability was assumed to be at most 40% (62–66.5% excreted unchanged in the feces)[6,7]. Nevertheless, it has been demonstrated that this value depends greatly on the administered doses: 94.4% after a single dose of 1 g and only 50% after daily doses of 8 g[8]. Then, to achieve therapeutic plasma level, high cumulative doses of mitotane were usually used during 3–5 months. With 1103 g (6–12 g/day; 119 ± 47 days) of micronized mitotane mixed with cellulose acetylphtalate[3], 58% success rate in obtaining 14 mg/L was obtained, whereas 100% was reported with 363 g (1–3 g/day, 3–5 months) of pure mitotane[4]. Although the studies are not completely comparable, it seems that mitotane formulation could impact bioavailability. Moreover, the administration of mitotane in chocolate, emulsion, and milk leads to higher plasma levels than tablets[9]. Generally, for lipophilic drugs like mitotane, increasing the solubility in aqueous media allows improving bioavailability after oral administration. Various formulation strategies are reported in the literature, including the use of surfactants, cyclodextrins, nanoparticles, solid dispersions, lipids, and permeation enhancers[10]. Concerning

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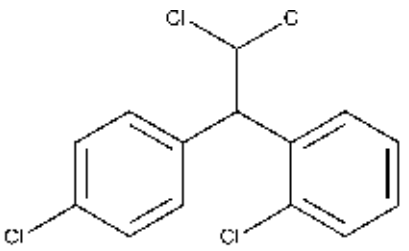

$C_{14}H_{10}Cl_4$
Mol. Wt.: 320.04

1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene

INN: mitotane

Figure 1. Chemical structure of mitotane.

mitotane, emulsions[11] and nanosuspensions[12] have been prepared with no further evaluation.

In recent years, one of the most popular approaches is the incorporation of the lipophilic component into self-microemulsifying drug delivery system (SMEDDS). The small oil droplets provide a large interfacial area for pancreatic lipase to hydrolyze triglycerides and release the drug and/or allow the formation of mixed micelles bile salts/drug. These SMEDDS are defined as isotropic mixtures of lipid, surfactant, cosurfactant, and drug that rapidly form a microemulsion after mixing with water. The digestive motility of the stomach and the intestine provides the agitation necessary for self-emulsification[13]. Factors such as the ability to form small droplets of oil (<100 nm) and the solubility of the drug in the oils and surfactants are important to achieve suitable SMEDDS. Moreover, regarding surfactants, they are known to improve the intestinal absorption by various mechanisms such as improved drug dissolution[14], increased intestinal epithelial permeability[15], increased tight junction permeability[16], and decreased/inhibited *p*-glycoprotein drug efflux[17].

Coenzyme $Q_{10,}$ an antioxidant[18], has reported a two-fold increase in the bioavailability of this poorly soluble compound when formulated as self-emulsifying drug delivery system (SEDDS). For simvastatin, a cholesterol-lowering agent, the pharmacokinetics in beagle dogs showed a 1.5-fold increase in bioavailability when compared with the conventional tablets[19]. Finally, a commercially available SMEDDS preparation with cyclosporine A (Neoral®), an immunosuppressant, is known to improve bioavailability in humans[20].

Because of its high lipophilicity, mitotane is also a good candidate for the formulation of microemulsions. Hence, the objective of this study was to develop and characterize SMEDDS of mitotane and to assess its bioavailability compared with that of conventional tablets (Lysodren®).

Materials and methods

Materials

Mitotane (*o*,*p*′-DDD or 1,1-dichlorodiphenyldichloroethane, Mr: 320.05 g/mol), Cremophor® EL, Tween® 20, and Tween® 80 were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Capryol® (propylene glycol monocaprylate) was a gift from Gattefossé (Gennevilliers, France). Myvacet® (acetylated monoglycerides) was purchased from Eastman (Kingsport, TN, USA), ethyloleate from Merck (Hohenbrunn, Germany), soya oil from Cooper (Melun, France), Miglyol® 812 (triglycerides of caprylic/capric acid) from Dynamit Nobel (Leverkusen, Germany), Captex® 355 (triglycerides of caprylic/capric acid) from Abitec Corporation (Janesville, WI, USA), and albumin was a gift from LFB (Courtaboeuf, France).

Solubility studies

The solubility of mitotane in oils and surfactants was determined as follows: 2 mL of each of these vehicles was added in screw-cap vials containing excesses of mitotane (i.e., 500 mg). After sealing, the mixture was heated in a shaking water bath (Memmert, Schwabach, Deutschland; 30°C, 48 hours, 60 strokes/min) to improve the solubilization. After equilibrium was achieved, the mixture was centrifuged at 1400 × *g* for 5 minutes and unsolubilized mitotane was discarded. Concentrations were determined by high-performance liquid chromatography (HPLC).

SMEDDS formulations

A series of mixtures were prepared with various ratios of Capryol®, Tween® 20, and Cremophor® EL. In each formulation, the amount of mitotane was set at 125 in 2000 mg (i.e., 6.25%) of vehicle phase. The oily solutions were prepared by dissolving the amount of mitotane in Capryol®; subsequently, Tween® 20 and Cremophor® EL were added and all components were mixed under magnetic stirring (500 rpm) until a transparent solution was obtained.

Pseudoternary phase diagrams of oil, surfactant, cosurfactant, and water were developed using titration method in the presence of the mitotane (125 mg, 6.25%). Three phase behavior systems were studied at various ratios of surfactant/cosurfactant: 1:0.5, 1:1, and 1:2 (w/w). The oily phase was added to such mixtures in different amounts: 5%, 10%, 20%, 30%, 40%, 50%, 60%, and 70% (w/w). Each mixture was then titrated by adding water up to clouding. Pseudoternary diagrams were constructed to identify the good self-emulsifying regions.

Emulsion droplet size analysis

The droplet size of the microemulsions was measured by photon correlation spectroscopy using a Zetasizer 3000® (Malvern Instruments, Orsay, France). Fifty microliters of the mixture was dispersed in 50 mL of purified water in a flask and gently mixed at 50 rpm. A mixture was defined to be SMEDDS if there is a formation of a crystal-clear microemulsion giving droplet sizes of less than 100 nm at ambient temperature when introduced to aqueous phase.

Release studies

The release of mitotane from SMEDDS was compared with that of the conventional formulation (Lysodren®) and a solution of mitotane in Capryol® (6.25%, w/w). A dialysis method was performed as follows: 62.5 mg of mitotane (SMEDDS of mitotane, Lysodren®, or the Capyol® solution) was poured in 20 mL of water and then instilled into the dialysis bag (10 kDa). Each bag was placed into a bath of 3000 mL of phosphate buffer (0.1 M, pH 6.8). As albumin was shown to improve the solubility of mitotane, 2% of bovine serum albumin (BSA) was added into the external solution to approach sink conditions. These preparations were placed under magnetic stirring (100 rpm) and aliquots were withdrawn during the release period (48 hours) and before HPLC analysis.

HPLC assays

Determination of mitotane was carried out by HPLC. Twenty microliters of sample was injected onto a C18 column (Nucleosil®, 5 µm, 0.46 mm, 25 cm; Macherey-Nagel, Eckbolsheim, France) using an autosampler (Spectra Physics AS1000). The mobile phase was a mixture of methanol and water (85:15, v/v) at a flow rate of 1.5 mL/min (Spectra Physics P1000XR; Thermo Electron S.A., Courtaboeuf, France). Detection was performed by UV spectrophotometry at 230 nm (Spectra Physics UV 1000), and peak surface was used for the quantification.

Studies of intestinal drug permeation

Jejunum from male Wistar (RjHan:WI, 400 g, 8–10 weeks old, Charles Rivers) was rapidly removed by surgery, washed with cold Krebs-Bicarbonate Ringer's (KBR) solution (114 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl, 1.1 mM $MgCl_2$, 1.25 mM $CaCl_2$, 1.65 mM Na_2HPO_4, 0.3 mM NaH_2PO_4, 25 mM $NaHCO_3$, 10 mM glucose, pH 7.4), and placed in a beaker aerated with a mixture of O_2/CO_2 (95:5). The jejunum was immediately stripped through the mucosal layer to remove the serosal and muscular layers under the dissection microscope. Mucosa was mounted between the two halves of an Ussing chamber (CHM8, 0.5 cm^2 WPI, Stevenage, UK).

The two compartments of the Ussing chamber were filled with cell culture Ringer and 2% of BSA at 37°C. Current electrodes (Electrode kit for Ussing chamber, WPI) were placed on each side: the transmucosal electrical resistance (TER) that reflects the integrity of the tissue was monitored during each experiment and tissue samples that showed TER < 30 Ω cm^2 were discarded[21].

As tablets of Lysodren® could not be inserted in the small chamber, the microemulsion of mitotane (SMEDDS of mitotane, 3 mM) was compared with small amounts of pure powder (3 mM). The crystals spread homogeneously in the donor compartment (mucosal side). The receiver compartment (serosal side) was filled with 2 mL of fresh medium. This compartment (2 mL) was entirely withdrawn after 20 minutes for quantification. The process (filling/withdrawal) was repeated every 20 minutes. Results are presented as cumulative values ($n = 3$).

Bioavailability studies

For single administrations, nine male rabbits, weighing 2.5–3 kg, were used. They were starved for 12 hours prior to the oral administration. Animals were allocated to three groups at random. The first group received mitotane in Capryol® (100 mg/kg, single dose orally), the second, SMEDDS of mitotane (100 mg/kg, single dose orally, mean particle size: 40 nm), and the third, the conventional form (Lysodren®) at 100 mg/kg.

Blood samples (1 mL) were collected from the ear vein into heparinized tubes at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 24 hours. Plasma samples were immediately separated from whole blood by centrifugation for 10 minutes at 1400 × *g* and stored at −18°C prior to analysis. Mitotane was analyzed by HPLC. Briefly, after frozen plasma samples were thawed at room temperature, 200 µL of plasma sample was diluted with 560 µL of methanol, mixed with vortex, and centrifuged at 1400 × *g* for 10 minutes. The methanolic supernatant was analyzed by HPLC. The calibration curve was obtained using various amounts (1, 2.5, 5, 10, 20 mg/L) in plasma and treated in the same conditions (correlation coefficient $r = 0.998$).

The area under plasma concentration–time curve ($AUC_{0-\infty}$) was estimated with Kaleidgraph 4.0. (Synergy Software) and the relative bioavailability (F) of SMEDDS to Lysodren® was calculated using the following equation:

$$F = \frac{AUC_{test}}{AUC_{reference}} \times 100\%. \quad (1)$$

Results and discussion

Solubility studies

The components used in self-emulsifying systems have to avoid the precipitation after dilution in the gut lumen in vivo. Therefore, these components should have very high solubilization capacities for the mitotane.

Results for solubility studies are presented in Table 1. Among the oils, Captex® and Capryol® provided the higher solubilization capacity (345 ± 13 and 282 ± 5 mg/mL, respectively). Tween® 20 and Cremophor® were the best surfactants (389 ± 7 and 409 ± 3 mg/mL, respectively). Hence, these components have been used for further experiments.

Pseudoternary phase diagram study

Phase diagrams were constructed in the presence of constant level of mitotane (6.25%) to obtain the optimum concentrations of oil, surfactant, and cosurfactant. The function of the surfactant and the cosurfactant is to reduce the interfacial energy and provide a mechanical barrier to coalescence: the decrease in the free energy required for the emulsion formation improves the stability of the microemulsion[22].

Preliminary diagrams were conducted with Captex®, Tween® 20 and Cremophor®, and mitotane but the mixtures did not allow us to obtain microemulsions areas. Mixtures of Capryol®, Tween® 20, and Cremophor® gave better results (Figure 2). In practice, the largest microemulsion formation area was obtained when the ratio of surfactant/cosurfactant (S/CoS) was equal to 1 (Figure 2b). Moreover, the effect of oil concentration on the droplet size distribution was investigated. The droplet size increased from 25 to 180 nm as the Capryol® concentration increased from 5% to 50%. When S/CoS is higher than 1, the size of the microemulsion region was slightly decreased. This could be explained by excessive cosurfactant, which could create instability of the interfacial film. Then, we selected S/CoS = 1 for an optimal formulation. Consequently, the selected formulation consisted of a mixture of Capryol®, Tween® 20, and Cremophor® (33:33:33), which was shown to spread rapidly in water, forming a clear and transparent microemulsion stable up to 3 days.

The droplet size of various formulations was studied. An increase of both surfactant and cosurfactant from 30% to 70% resulted in a decrease in the mean particle size. A smaller droplet size (40 nm) was observed when Capryol®, Tween® 20, and Cremophor® EL were in equal proportion. Tween® 20 and Cremophor® EL probably increased the penetration of water into the bulk oil, causing interfacial disruption and ejection of droplets into the bulk aqueous phase as reported by Pouton[23]. In our study, we investigated the effect of mitotane on droplet size. As drug loading increased from 6.25% to 25% the droplet size remained unchanged (Figure 3).

Above 25%, the mean size increased strongly with the drug concentrations probably as a consequence of undissolved mitotane in the formulation, which affected the apparent droplet size.

Table 1. Solubility of mitotane in various vehicles.

Vehicle	Solubility of mitotane (mg/mL)
Myvacet®	71 ± 16
Capryol®	282 ± 5
Ethyloléate	114 ± 5
Soya oil	16 ± 10
Miglyol® 812	232 ± 10
Captex® 355	345 ± 13
Tween® 20	389 ± 7
Tween® 80	226 ± 9
Cremophor® EL	409 ± 3
Human albumin (2% in water)	0.036 ± 0.010

Concentrations were determined as described in 'Materials and methods' (mean ± SD; $n = 3$).

In vitro release study

Release studies were conducted for SMEDDS (Capryol® 33%, Tween® 20 33%, and Cremophor® EL 33%) mitotane in Capryol® and Lysodren® by a dialysis method. In our conditions, the solubility of mitotane in the external compartment (2% of BSA) was 0.036 mg/mL, and the study could be conducted near sink conditions. Nevertheless, as BSA is not able to pass in the bag, the release occurs in pure water.

As shown in Figure 4, the release of mitotane from SMEDDS was higher and faster than from Lysodren® (62 ± 3% versus 12 ± 5%, after 48 hours), confirming the solubilization power of microemulsions. On the contrary, the Caproyl® solution did not release significant amounts of mitotane (3.1 ± 0.3 %). In this latter formulation, the lack of surfactant/cosurfactant avoids a good dispersion of the drug, and the partition occurs merely following the oil/water partition rules. This formulation was not used for further studies.

Studies of intestinal drug permeation

In this experiment, mitotane is directly added (donor compartment) in the culture Ringer of the Ussing chamber. As hypothesized, the SMEDDS of mitotane passed through the intestinal barrier much faster than the suspension (14.85 ± 0.8 versus 3.03 ± 0.2 µmol/cm^2, Figure 5). This can be partly explained by

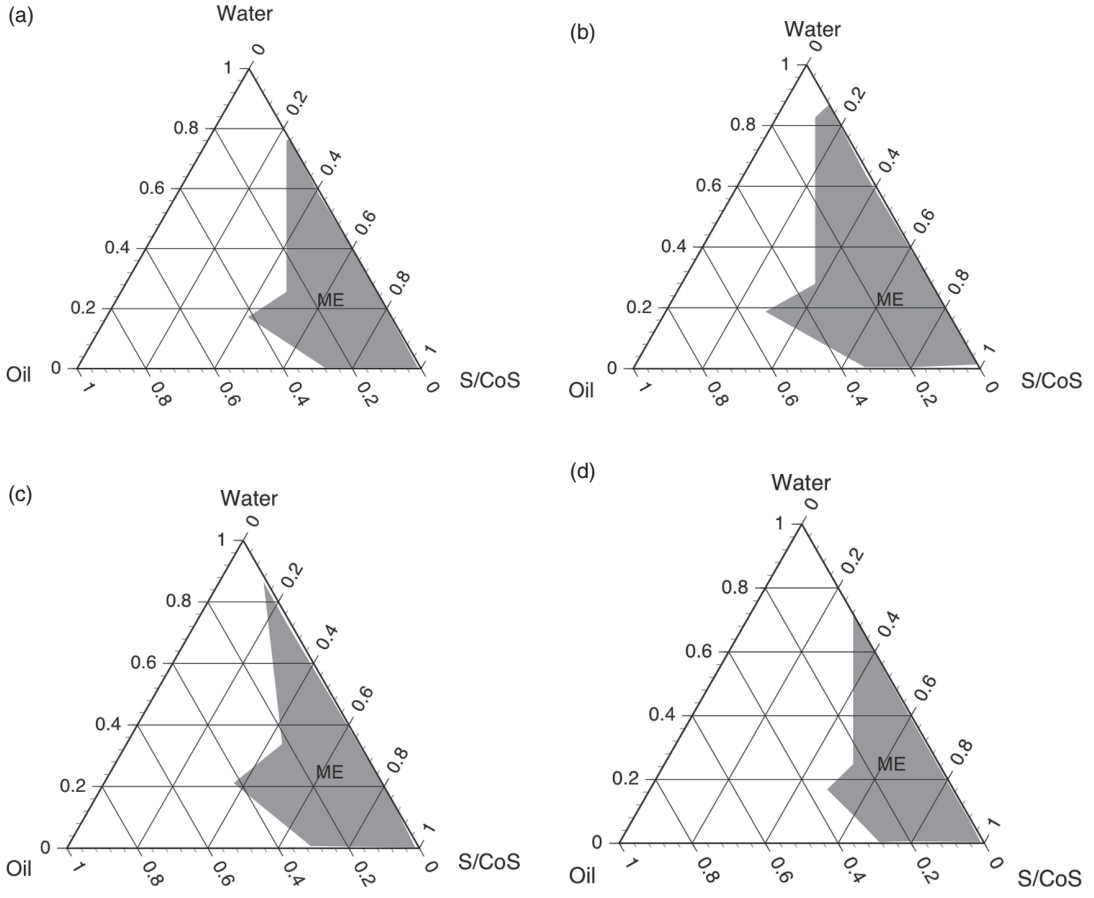

Figure 2. Pseudoternary phase diagram of SMEDDS. (a) Tween 20/Cremophor = 1:0.5; (b) Tween 20/Creomphor = 1:1; (c) Tween 20/Cremophor = 1:2; (d) Tween 20/Cremophor = 1:3. ME, microemulsion zone.

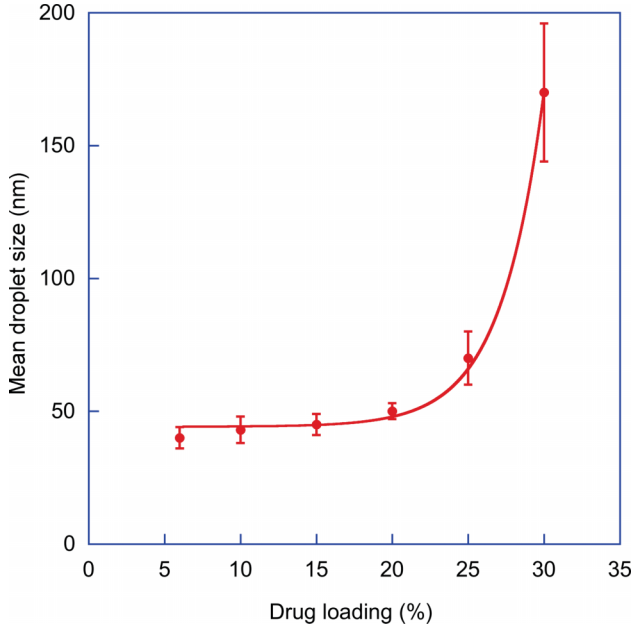

Figure 3. Effect of mitotane on the mean droplet size.

the very poor solubility of mitotane in the aqueous medium even though the BSA (2%) improves the solubility (Table 1). Moreover, H. Araya explained that drugs entrapped in o/w microemulsions are released in the mucin layer, without passing through the route of the mixed micelle formation by bile, and thus permeates the intestinal membrane efficiently[24]. Hence, SMEDDS can be regarded as drug carriers that are able to deliver mitotane to the intestine barrier in a more efficient way than the conventional formulations.

In vivo studies

The in vivo pharmacokinetics of mitotane in Capryol®, mitotane in SMEDDS (Capryol® 33%, Tween® 20 33%, and Cremophor® EL 33%), and Lysodren® were investigated in rabbits. Figure 6 shows the plasma concentrations of mitotane after single oral administrations of the three formulations. Pharmacokinetic parameters are given in Table 2. The C_{max} and $AUC_{0-\infty}$ of the SMEDDS were significantly higher than those of mitotane in Capryol® and Lysodren® (i.e., the relative bioavailability

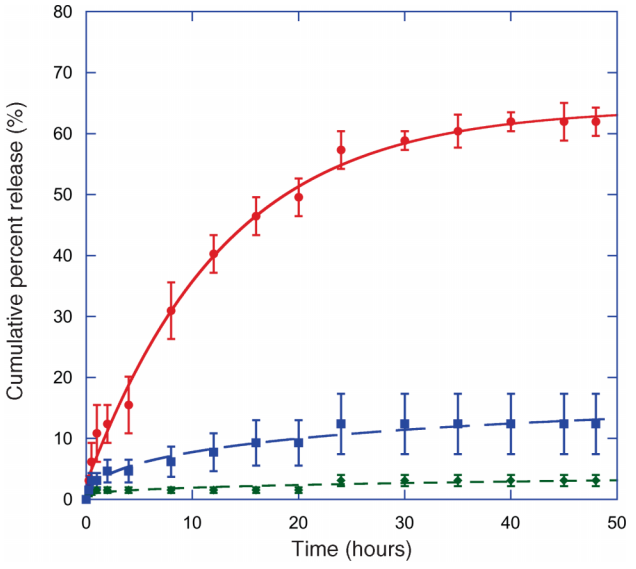

Figure 4. Cumulative percent of release of mitotane by dialysis. -●-, from SMEDDS of mitotane; -■-, from Lysodren®; -◆-, from mitotane in Capryol®. Medium: phosphate buffer (0.1 M, pH 6.8) with 2% of BSA. Symbols represent means of three experimental determinations. Vertical lines indicate mean ± SD.

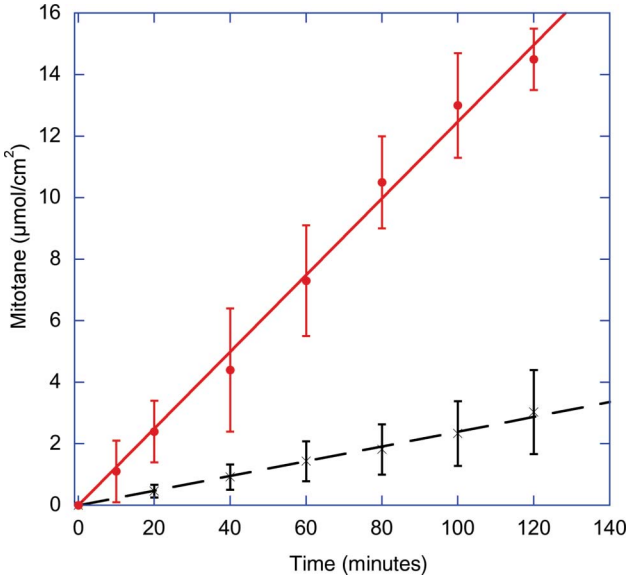

Figure 5. Mucosal-serosal transepithelial flux of mitotane across rat jejunum at 37°C. The drug was added as a suspension of rough powder (3 mM, -x-) or as a microemulsion of mitotane (SMEDDS of mitotane, 3 mM, -●-) in the donor compartment (mucosal side). Symbols represent means of three experimental determinations. Vertical lines indicate mean ± SD.

of SMEDDS was 3.4 with Lysodren® as reference). This value can be compared with the 5.1-fold improvement of release (63.3% versus 12.5% after 48 hours, Figure 4) and with the 4.9-fold improvement of absorption (Figure 5). As previously said, the bioavailability

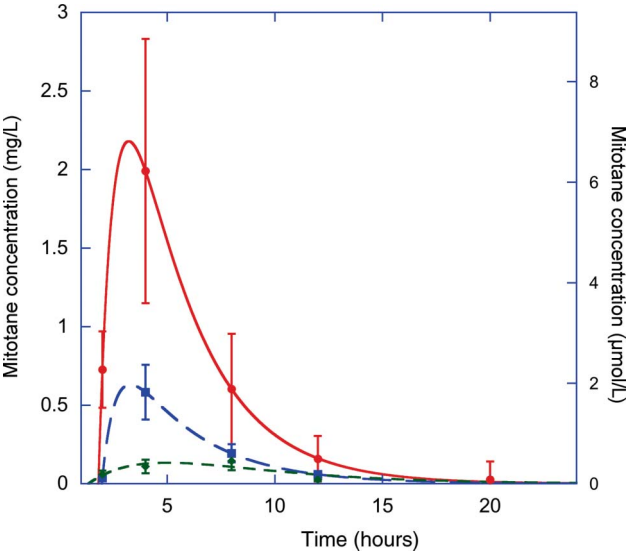

Figure 6. Plasma concentration of mitotane after administration of 100 mg/kg (single dose, orally) of mitotane (-●-, SMEDDS of mitotane; -■-, Lysodren®; -◆-, mitotane in Capryol®). Symbols represent means of three experimental determinations. Vertical lines indicate mean ± SD.

Table 2. Relative bioavailability and pharmacokinetics parameters of mitotane after oral administration of SMEDDS of mitotane, mitotane in Capryol®, and Lysodren® ($n = 3$).

	SMEDDS of mitotane	Capryol® solution of mitotane	Tablets (Lysodren®)
$AUC_{0-\infty}$ (mg·h/L)	10.5 ± 0.9	1.3 ± 0.02	3.1 ± 0.3
C_{max} (mg/L)	2.2 ± 0.4	0.13 ± 0.06	0.63 ± 0.19
T_{max} (hours)	3.2 ± 0.03	5.0 ± 0.14	3.3 ± 0.14
Relative bioavailabilty	3.4	0.42	1

depends greatly on the administered dose, on the formulation, and probably on the animal species. Hence, it would be abusive to compare too accurately our results with those obtained in other studies with various formulations (tablets, capsules, etc.) in humans[8]. Nevertheless, compared with other compounds, the improvement is very important. Actually, the well-known SMEDDS of cyclosporin have been shown to increase the bioavailability by a factor of 1.6[25] and as a function of the dose up to 2.39-fold[26].

Conclusion

The bioavailability of mitotane is known to be very low and therapeutic levels are achieved only after 3–5 months of high amounts (6–12 g/day) of drug. Moreover, pronounced interindividual variation in plasma

concentrations after administration of oral doses has been reported. The daily mitotane dose explained only 35% of the variability between patients, suggesting that the formulation and food bolus[3] are of most importance. These variations may impact the treatment efficiency.

An optimal SMEDDS formulation of mitotane (33% of Capyol, 33% of Tween 20, and 33% of Cremophor EL) showed a 3.4-fold improvement of the bioavailability compared to Lysodren® and can now be considered as a very good candidate to optimize the administration of mitotane. Reducing the doses from 10 to 2 or 3 g would undoubtedly make the treatment more acceptable for the patients. Nevertheless, as major side effects of mitotane include gastrointestinal effects, clinical studies will have to determine the impact of this formulation on this side effects and on the compliance of the patients.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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