STUDIES ON LIPIDOMIMETIC DERIVATIVES OF α -DIFLUOROMETHYLORNITHINE (DFMO) TO ENHANCE THE BIOAVAILABILITY IN A *Trypanosoma b. brucei* MURINE TRYPANOSOMIASIS MODEL

LOISEAU P.M.*, CZOK M.**, CHAUFFERT O.*, BOURASS J.*** & LETOURNEUX Y.***

Summary :

DFMO, a trypanostatic drug, presents a satisfactory intestinal absorption but its elimination from the blood is rapid so that high doses are necessary to obtain a therapeutic effect. In this study, we propose a strategy to enhance the bioavailability of DFMO by using lipidomimetic derivatives. Three lipidomimetic DFMO derivatives called O-DFMO, S-DFMO and Chol-DFMO were designed to reach easily the plasma and to be cleaved preferentially by plasma esterases progressively liberating free DFMO. Chol-DFMO only could be cleaved partially whereas the other compounds appeared to be stable in a reconstituted intestinal medium and mouse plasma. Nevertheless, the use of DFMO derivatives in *T. b. brucei* experimental chemotherapy appeared as an interesting approach. Thus, O-DFMO was trypanocidal *in vitro* whereas DFMO, the active principle, was only trypanostatic. Nevertheless, this compound did not release DFMO in mouse blood as expected and acted therefore not as a prodrug. Oral treatment using low doses of compound O-DFMO was able to cure 40 % mice while the active principle (eflornithine) administered at 50 fold higher molarity failed to cure any mice. This indicates that compound O-DFMO acts by a specific mechanism which remains to be investigated. S-DFMO was less active and Chol-DFMO had no *in vitro* activity but released small amounts of DFMO in mice, however, too slight to obtain a therapeutic effect.

KEY WORDS : α -difluoromethylornithine, lipidomimetic derivatives, *Trypanosoma brucei brucei*, antitrypanosomal compounds.

Résumé : ÉTUDE DE DÉRIVÉS LIPIDOMIMÉTIQUES DE L' α -DIFLUOROMÉTHYLORNITHINE (DFMO) POUR EN AUGMENTER LA BIODISPONIBILITÉ DANS UN MODÈLE DE TRYPANOSOMOSE MURINE À *Trypanosoma b. brucei*

La DFMO, agent trypanostatique, présente une absorption intestinale satisfaisante mais son élimination sanguine rapide impose des doses élevées pour obtenir un effet thérapeutique. Nous proposons, dans cette étude, une stratégie pour augmenter la biodisponibilité de la DFMO en utilisant des dérivés lipidomimétiques. Trois dérivés lipidomimétiques de la DFMO appelés O-DFMO, S-DFMO et Chol-DFMO ont été conçus pour gagner facilement le sang et être clivés préférentiellement par les estérases plasmatiques en libérant de la DFMO de façon progressive. Seul le Chol-DFMO a été clivé partiellement tandis que les autres composés se sont montrés stables aussi bien en milieu intestinal reconstitué que dans le plasma de souris. Malgré cela, l'utilisation de dérivés de la DFMO en chimiothérapie expérimentale à *T. b. brucei* a constitué une approche intéressante. Ainsi, le O-DFMO a montré une activité trypanocide *in vitro* tandis que la DFMO, le principe actif, n'était que trypanostatique. Néanmoins, ce composé n'a pas libéré de DFMO dans le sang des souris comme on s'y attendait; il n'a donc pas un comportement de prodrogue. Un traitement par voie orale avec de faibles doses de O-DFMO a guéri 40 % des souris alors que le principe actif (éflornithine) administré à une molarité 50 fois supérieure n'a guéri aucune souris. Le mécanisme d'action spécifique du composé O-DFMO reste à étudier. Le composé S-DFMO s'est montré moins actif. Quant au Chol-DFMO, il n'a présenté aucune activité *in vitro* mais a libéré de petites quantités de DFMO chez la souris, trop faibles cependant pour observer un effet thérapeutique.

MOTS CLÉS : α -difluorométhylornithine, dérivés lipidomimétiques, *Trypanosoma brucei brucei*, composés trypanocides ou trypanostatiques.

INTRODUCTION

The newest drug to be approved by the U.S. Food and Drug Administration for use against African trypanosomiasis is DFMO (α -difluoromethylornithine). DFMO is a catalytic irreversible inhi-

bitor of ornithine-decarboxylase (ODC), a key enzyme of polyamine biosynthesis (Haegele *et al.*, 1981). Although this compound was originally designed as an antitumor agent, it has proven to be highly effective against *Trypanosoma brucei gambiense* infections in man (Doua *et al.*, 1988; Van Nieuwenhove, 1992). Monotherapy with intravenous DFMO of 400 mg/kg/day for five to six weeks led to disappearance of parasites from the cerebrospinal fluid and the reversal of clinical signs. Furthermore, a recent report deals with the synergism between melarsoprol and DFMO in Gambian trypanosomiasis (Simarro & Ndong Asumu, 1996). Although combinations of DFMO with

* Biologie et Contrôle des Organismes Parasites,

** Chimie Analytique, Faculté de Pharmacie, Université de Paris XI, 92296-Châtenay-Malabry Cédex, France.

*** Laboratoire SESNAB, Université de La Rochelle, 17026 La Rochelle Cedex, France.

Correspondence: Dr. P. Loiseau; Tél. : 33 (0) 1 46 83 55 54 – Fax: 33 (0) 1 46 83 55 57 – E-mail: Philippe.Loiseau@cep.u-psud.fr

either suramin or melarsen oxide were found to be effective against acute laboratory model infections of *Trypanosoma brucei rhodesiense* (Clarkson *et al.*, 1984; Jennings, 1992; Bacchi *et al.*, 1994), chemotherapy for East African sleeping sickness remains unreliable: drug resistance to arsenical drugs is common and clinical strains refractory to DFMO and diamidines have also been documented (Bayles *et al.*, 1989). A mean to limit the risk of resistance is to enhance the bioavailability of the active principle in the host in order to limit the duration of treatment and massive dosages. DFMO presents a satisfactory intestinal absorption in rats (Levin *et al.*, 1983). After a single oral dose of 200 mg/kg, the maximum plasma concentration is 100-200 nmol/ml; this concentration is suitable to inhibit ornithine decarboxylase *in vitro* but the DFMO elimination is rapid since the plasma half life is about 1-2 h. These data explain that high dosages are necessary to obtain a therapeutic effect. The oral activity of DFMO is an advantage for mass treatment. In this field, we propose here a strategy to maintain DFMO plasma concentration using lower doses of drug. The use of lipidomimetic derivatives has been previously developed with success in the field of antifilarials using experimental filariasis model (Deverre *et al.*, 1989; Elkihel *et al.*, 1994; Loiseau *et al.*, 1994). Such derivatives, as prodrugs, can be considered as controlled release systems avoiding rapid elimination of the active principle. The hydrolysis of the prodrugs should be slow to maintain a therapeutic level of plasma drug concentration. This paper reports the *in vitro* and *in vivo* trypanocidal effects of several DFMO derivatives, their *in vitro* stability in the presence of esterases and their potential to liberate DFMO *in vivo* by a kinetics study.

MATERIALS AND METHODS

CHEMICALS

α -difluoromethylornithine hydrochloride (eflornithine) was supplied by Merrell Dow Center, Strasbourg, France.

Three prodrugs have been synthesized:

- 1,3-dipalmitylether-2-acetyl-glycerol-DFMO di-trifluoroacetate briefly called O-DFMO. This prodrug possesses an ether bond between palmityl groups and

Fig. 1. – Chemical structure of O-DFMO: M.W. = 990.3 g.

glycerol. The chemical structure of O-DFMO is reported in Figure 1.

- 1,3-dipalmitylthioether-2-acetyl-glycerol-DFMO di-trifluoroacetate briefly called S-DFMO. This prodrug possesses a thioether bond between palmityl groups and glycerol. The chemical structure of S-DFMO is reported in Figure 2.

Fig. 2 – Chemical structure of S-DFMO: M.W. = 1022.3 g.

- Cholesterol-DFMO di-trifluoroacetate briefly called Chol-DFMO. Its chemical structure is reported in Figure 3.

Fig. 3. – Chemical structure of Chol-DFMO: M.W. = 836.8 g.

The ligand called 1,3-D-O was also tested *in vitro* and *in vivo* to assess its inactivity on parasites. The chemical structure of 1,3-D-O is reported in Figure 4.

Fig. 4. – Chemical structure of 1,3-D-O: M.W. = 598 g.

THE ANIMALS

Female CD1 mice, 18-20 g (Charles River, Cléon, France) were used for the experiments.

THE PARASITE

Trypanosoma brucei brucei CMP (Châtenay-Malabry Parasitologie) was obtained from the Institut Pasteur, Paris in 1973. This strain was kept frozen in liquid nitrogen, and an aliquot was passaged in female CD1 mice before the experiments.

Trypanosoma brucei brucei GVR 35/Cl.2 was kept frozen in liquid nitrogen and was kindly supplied by Dr. F.W. Jennings

(Glasgow, U.K.). This strain had been derived from trypanosomes isolated from a wild beast in the Serengeti (Tanzania) in 1966 (Serengeti/66/SVRP/10). It produces a chronic infection in mice, allowing them to survive for at least 30 days.

IN VITRO EVALUATION

Trypanosomes harvested from infected mice were cultured in a semi-synthetic medium allowing growth and multiplication for 48 heures without loss of infectivity. The DIIT (Drug Incubation Infectivity Test) was used for compound evaluation (Kaminsky *et al.*, 1990). The culture medium used throughout the study consisted of Minimum Essential Medium (MEM) with Earle's salts and L-glutamine supplemented with 25 mM HEPES, 1g/l additional glucose, 2.2 g/l NaHCO₃, 10 ml/l MEM non essential amino-acids (100 ×), 0.2 mM 2-mercaptoethanol, 2 mM sodium pyruvate, 0.1 mM hypoxanthine, 0.016 mM thymidine, 15 % heat-inactivated horse serum, 100 UI penicillin/ml and 100 µg streptomycin/ml. The culture medium was sterilized by filtration. Blood was collected aseptically from the retro-orbital sinus of infected mice having about 10⁸ trypanosomes/ml blood, diluted with medium and kept on ice. The trypanosomes were isolated from this suspension by centrifugation at 900 g for 10 min at 4 °C; the supernatant was discarded and trypanosomes were at the top of the pellet. The number of trypanosomes was determined by haemocytometer counting and adjusted to 2 × 10⁵ parasites/ml.

The bloodstream forms of *T. b. brucei* CMP were maintained *in vitro* without loss of infectivity for 48 h in the dark at 37 °C in 5 % CO₂. The screening was performed in 24-well tissue culture plates (Falcon No 3047). Each well received 1.4 ml culture medium. The wells were then inoculated with 2 × 10⁴ trypanosomes in 0.1 ml. Then 10 µl of an adequate dilution/ suspension of eflornithine or derivatives in water/DMSO was added to each well. Drug concentrations from 4 mM to 0.5 µM were evaluated in duplicate. The number of motile parasites was determined by haemocytometer counting, using an optical microscope. The effect on multiplication was assessed both visually and *in vivo* by intraperitoneal injection into naive mice of 0.2 ml of treated trypanosome suspension withdrawn from the wells. The mice were checked for parasitaemia weekly for 30 days.

CYTOTOXICITY ASSAY OF THE COMPOUNDS ON MOUSE PERITONEAL MACROPHAGES

Mouse peritoneal macrophages, 5 × 10⁵ cells per well, were plated on sterile 96-well plates (Nunc, Paris, France), incubated for 4 hours in an atmosphere of 5 % CO₂ and 95 % air, at 37 °C. Adherent peritoneal macrophages were washed twice with complete medium and

reincubated for 24 hours. Then, 200 µl of medium containing different concentrations of DFMO or its derivatives were added. The viability of cells was determined by the dimethyldiphenyltetrazolium bromide (MTT) test (Mosmann, 1983) after 24 hours of incubation.

STABILITY OF THE DFMO DERIVATIVES

This study was performed in intestinal reconstituted medium, in medium for trypanosome culture and in infected and noninfected mouse plasma.

Preparation of the DFMO derivatives suspensions:

1 mg of DFMO derivative was dissolved in 0.1 ml of CH₂Cl₂. 0.1 ml of an aqueous solution containing 0.5 % Tween 80 was added. The two phases were mixed by sonication. The suspension was maintained at 37 °C for 20 min to assess the emulsion stability.

The stability of DFMO and its derivatives was studied by thin layer chromatography, after *in vitro* incubations at 37 °C in reconstituted intestinal medium, in medium for trypanosome culture and in infected and noninfected mouse plasma.

The medium was prepared as follows: 6.8 g of KH₂PO₄ were dissolved in 250 ml water. Then 190 ml of 0.2 N NaOH and 400 ml water were added. 10 g of porcine pancreatin (FU, USP; Merck, Nogent sur Marne, France) including 350 U-FIP proteases/g, 7500 U-FIP lipase/g and 7500 U-FIP amylase/g were mixed with the previous solution. The solution was filled up to 1,000 ml with water and the buffer pH was adjusted to 7.5 ± 0.1 using 0.2N NaOH.

The suspension of DFMO derivative previously prepared was added to 1 ml of intestinal reconstituted medium or medium for trypanosome culture or mouse plasma. The assays were carried out with samples gathered after 15, 30, 60, 120, 180 and 240 minutes of incubation. The activities of enzymes in intestinal medium or medium for trypanosome culture or in plasma were blocked for each sample by diluting the sample with two HCl solution volumes at pH 3. The samples were then freeze-dried, lyophilized and analyzed by thin layer chromatography (Loiseau *et al.*, 1994).

PLASMA KINETICS OF DFMO

A previous study demonstrated that a chronic trypanosomiasis in mice modifies the plasma kinetics of DFMO so that we decided to work with the *T. b. brucei* GVR 35 strain to observe the effect of the chronic trypanosomiasis on the DFMO derivatives bioavailability (Loiseau *et al.*, 1997b). Plasma kinetics was performed on 21-day infected and noninfected mice after treatment at 2 mmol/kg with DFMO and its derivatives by oral route. Blood samples for drug level detection were collected each 30 min for the first 4 h and each

hour for 10 h after treatment with DFMO derivatives. After anesthesia using carbon dioxide, blood was collected from the retro-orbital sinus into citrated glass pipettes and was centrifuged at 2,200 g for 15 min. Plasma samples were then deproteinized using perchloric acid (100 v/1 v) and centrifuged at 2,200 g for 15 min. The supernatant was kept for assay. DFMO was assayed in plasma following the HPLC method described by Loiseau *et al.*, 1997b.

IN VIVO EVALUATION ON *T. B. BRUCEI* CMP

The protocol for *in vivo* evaluation has been previously described (Loiseau *et al.*, 1993). Mice were infected intraperitoneally with 10^3 bloodstream trypomastigotes suspended in 0.1 ml phosphate buffered saline, pH 7.2. Ten infected mice were used as controls and received only excipient. The other mice were treated by oral route with the DFMO derivatives suspended in 1 % carboxymethylcellulose in sterile water. A drop of Tween 80 was previously added to the powder. The treatment was performed two times per day for six days using at least six mice per dose. The trypanocidal activity was evaluated by the mean survival time of treated mice for each dose. Treatment was considered as successful when the mean survival time exceeded thirty days and the mice remained aparasitaemic.

RESULTS

IN VITRO EVALUATION USING *T. B. BRUCEI* CMP

This fast strain of *T. b. brucei* allows to obtain rapid responses concerning the therapeutic effect of DFMO and its derivatives. The trypan-

osome culture in the semi-defined acellular medium allowed growth from an initial trypanosome number of 5×10^4 per well to 20×10^4 after a 48 h incubation time. Afterwards, the trypanosome number reached a plateau for 12 h and then dropped.

Eflornithine was trypanostatic at 4 and 2 mM after 12 and 24 h of incubation respectively (Fig. 5). The injection of treated trypanosomes to mice did not provoke any infection. Thus, the living parasites have entirely lost their potential for multiplication. A concentration of 1 mM exhibited a trypanostatic effect after 48 h, but after 24 h, this effect was incomplete since the injection of treated parasites was fatal for one mouse (Table I). The eflornithine IC_{50} determined after 12, 24, 36 and 48 h was 1,057, 484, 467 and 450 μ M respectively. No morphologic modification was noted during these experiments.

The DFMO derivatives could be assessed up to 200 μ M since a precipitate appeared in the wells when higher concentrations were used.

O-DFMO killed all parasites at 50 μ M after 24 h of incubation (Fig. 6). At 10 μ M, the trypanocidal effect was complete after 36 h. A 5 μ M concentration was trypanostatic after 24 h of incubation and the parasites treated at this concentration were not able to infect mice. The 50 % lethal concentration (LC_{50}) was 25.1, 6.2 and 4.2 μ M after 12, 24 and 36 h incubation time respectively.

S-DFMO was less active than O-DFMO since 200 μ M was necessary to obtain a trypanocidal effect after 48 h (Fig. 7). All the parasites were lysed and the injection of treated parasites into mice was not infective. A concentration of 50 μ M was trypanostatic after 48 h of incubation and the treated parasites were not infective whereas mice got infected when the incubation time was reduced at 24 h.

Chol-DFMO exhibited no *in vitro* action at 200 μ M.

Fig. 5. – *In vitro* trypanostatic activity of eflornithine on *Trypanosoma brucei brucei* CMP. The protocol was as described in Materials and methods ($n = 3$).

CYTOTOXICITY ASSAY OF THE COMPOUNDS ON MOUSE PERITONEAL MACROPHAGES

Eflornithine and Chol-DFMO had no cytotoxic action on mouse peritoneal macrophages at 2 mM while the LC₅₀ (lethal concentration 50 %) of O-DFMO and S-DFMO were 0.935 and 1.340 mM respectively after 48 h of incubation.

STABILITY OF THE PRODRUGS

in intestinal reconstituted medium
and in medium for trypanosome culture

O-DFMO, S-DFMO and Chol-DFMO were stable in the intestinal reconstituted medium and in culture medium for trypanosomes. In plasma, O-DFMO and S-DFMO

Compound	<i>In vitro</i> evaluation			<i>In vivo</i> evaluation		
	Concentration (µM)	Incubation time (h)	Presence of living trypanosomes*	Number of inoculated mice**	Number of survival days post-inoculation	Activity***
DFMO	4,000	24	+	4	>30, >30, >30, >30	4/4
		48	+	4	>30, >30, >30, >30	4/4
	2,000	24	+	4	>30, >30, >30, >30	4/4
		48	+	4	>30, >30, >30, >30	4/4
	1,000	24	+	4	6, >30, >30, >30	3/4
		48	+	4	>30, >30, >30, >30	4/4
	500	24	+	4	5, 5, 6, >30	1/4
		48	+	4	6, >30, >30, >30	3/4
	50	24	+	4	4, 5, 5, 7	0/4
		48	+	4	4, 4, 6, 6	0/4
O-DFMO	200	24	-	4	>30, >30, >30, >30	4/4
		48	-	4	>30, >30, >30, >30	4/4
	50	24	-	4	>30, >30, >30, >30	4/4
		48	-	4	>30, >30, >30, >30	4/4
	10	24	+	4	>30, >30, >30, >30	4/4
		48	-	4	>30, >30, >30, >30	4/4
	5	24	+	4	>30, >30, >30, >30	4/4
		48	+	4	>30, >30, >30, >30	4/4
	1	24	+	4	5, 7, 6, 7	0/4
		48	+	4	7, >30, >30, >30	3/4
	0,5	24	+	4	4, 5, 5, 7	0/4
		48	+	4	4, 4, 5, 6	0/4
S-DFMO	200	24	+	4	>30, >30, >30, >30	4/4
		48	-	4	>30, >30, >30, >30	4/4
	50	24	+	4	4, 5, 6, 6	0/4
		48	+	4	>30, >30, >30, >30	4/4
	10	24	+	4	4, 4, 5, 5	0/4
		48	+	4	4, 5, 5, 5	0/4
Chol-DFMO	200	24	+	3	4, 5, 5	0/3
		48	+	4	5, 5, 5, 6	0/4
1,3-D-O	200	24	+	4	4, 4, 5, 5	0/4
		48	+	4	4, 5, 6, 6	0/4
Controls Excipient	****	24	+	5	4, 5, 5, 5, 5	0/5
		48	+	5	4, 4, 5, 5, 6	0/5

* Observation with inverted microscope.

** Mice were inoculated by intraperitoneal route with 0.2 ml of the trypanosome suspension incubated with the compounds to be tested for 24 or 48 h.

*** Activity was defined as the ratio of mice without parasites 30 days post-inoculation to the total number of inoculated mice per batch.

**** 0,2 ml of medium for trypanosome culture including the excipient was inoculated into mice by intraperitoneal route.

Table I. – Combined *in vitro* and *in vivo* evaluation of DFMO and its derivatives on *T. b. brucei* CMP.

Fig. 6. – *In vitro* activity of O-DFMO on *Trypanosoma brucei brucei* CMP bloodstream forms ($n = 3$).

Fig. 7. – *In vitro* activity of S-DFMO on *Trypanosoma brucei brucei* CMP bloodstream forms ($n = 3$).

were stable whereas Chol-DFMO was partially degraded in both infected and noninfected mouse plasma.

PLASMA KINETICS OF DFMO

DFMO was assayed in plasma after a treatment by oral route at 2 mmol/kg with DFMO and its derivatives. After a treatment with O-DFMO and S-DFMO, no DFMO level could be detected in plasma of 21-day infected and noninfected mice. Plasma samples were collected until 14 h following the treatment. A treatment carried out in the same conditions with Chol-DFMO allowed to detect small amounts of DFMO

around the detection level (5-6 nmol/ml) in the plasma from noninfected mice and 13-15 nmol/ml in plasma from infected mice one hour after treatment by oral route.

IN VIVO EVALUATION

Eflornithine administered *ad libitum* in drinking water at 2 % for six days cured all the mice and no parasite was found 30 days post-infection. The parasitemia of these mice remained negative throughout the experiment. Nevertheless, a treatment per os at 2g/kg/day (8,474 µmoles/kg) for six days gave a mean survival time of seven days comparatively to control mice

treated only with excipient. Some trypanosomes had a rounded form and their size increased to 25-30 μm . A treatment carried out in the same conditions but with 84.7 $\mu\text{mol/kg}$ was without effect on the development of murine trypanosomiasis (Table II).

Concerning the DFMO derivatives, O-DFMO was able to cure 4/10 mice after a treatment at 169.4 $\mu\text{mol/kg}$ whereas DFMO cured 0/10 mice after a dose at 8,474 $\mu\text{mol/kg}$. S-DFMO and Chol-DFMO were totally inactive *in vivo*. The ligand 1,3-D-O was also inactive at 169.4 $\mu\text{mol/kg}$ (Table II).

DISCUSSION AND CONCLUSION

In vitro, two DFMO derivatives (O-DFMO and S-DFMO) were able to kill the trypanosomes whereas DFMO itself only inhibited parasite multiplication. Chol-DFMO was without effect even using high concentrations. This toxic effect of DFMO derivatives toward the parasites was also detected using a cytotoxicity assay on mouse peritoneal macrophages. All the DFMO derivatives remain stable in intestinal reconstituted medium. This result is in favour of an intestinal absorption of the compounds without structural modification. In plasma, Chol-DFMO alone is partially degraded. This observation is confirmed *in vivo* since small amounts of DFMO could be detected after a treatment with Chol-DFMO. These data show

that Chol-DFMO only can be considered as a prodrug of DFMO because of its ability to liberate the active principle in the plasma. Nevertheless, the DFMO amounts released from Chol-DFMO in plasma (13-15 nmol/ml) were ten times too low to observe a therapeutic effect (100-200 nmol/ml). Chol-DFMO was therefore resistant to intestinal esterases but was cleaved slowly in plasma since the maximum concentration of DFMO appeared 1 h after treatment with Chol-DFMO whereas the maximum concentration of DFMO appeared 30 min after a treatment with DFMO by oral route. *In vitro* experiments did not exhibit degradation products from O-DFMO and S-DFMO and *in vivo* data demonstrate that there are no intestinal and plasma enzyme systems able to cleave these compounds. O-DFMO and S-DFMO should act themselves by a mechanism of action that remains to be investigated.

Previous pharmacokinetic studies on DFMO in human have shown that maximum plasma concentrations were reached 4 h after treatment with oral dose of 10 mg/kg (Haeghele *et al.*, 1981). DFMO is well absorbed with an average $t_{1/2}$ of absorption of 74 min. The kinetics in mice is more rapid since the C_{max} appears 30 min after the treatment (Loiseau *et al.*, 1997b).

In the recent years, lipidomimetic compounds have been developed to obtain prodrugs able to modify the pharmacokinetics of antiparasitic compounds such as niclosamide, closantel or GABA (Elkihel *et al.*, 1994;

Compound	Treatment regimen		Duration (days)	Mice number	MST after infection (days)	No cured/ treated	Activity
	$\mu\text{mol/kg}$	g/kg					
DFMO*	<i>ad libitum</i> **		$\times 6\text{d}$	10	>30	10/10	++
	8,474	2	2/d $\times$ 6	10	11.5 ± 2.2	0/10	+
	84.7	0.02	2/d $\times$ 6	10	4.5 ± 0.5	0/10	0
O-DFMO***	169.4	0.168	2/d $\times$ 6	10	7.4 ± 2.5	4/10	+
	84.7	0.084	2/d $\times$ 6	6	4.6 ± 0.8	0/6	0
S-DFMO***	169.4	0.173	2/d $\times$ 6	10	4.5 ± 0.7	0/10	0
Chol-DFMO***	169.4	0.142	2/d $\times$ 6	10	4.7 ± 0.6	0/10	0
1,3-D-O***	169.4	0.101	2/d $\times$ 6	10	4.6 ± 0.3	0/10	0
Control: 1 % CMC + Tween 80	—	—	2/d $\times$ 6	6	4.5 ± 0.4	0/6	0

Mice were infected by intraperitoneal route with 1,000 trypanosomes. Oral treatment was commenced two hours post-infection with a 0.2 ml volume.

* Eflornithine.

** Eflornithine was dissolved in water (2 %).

*** DFMO derivatives and 1,3-D-O were administered as an emulsion in a 1 % carboxymethylcellulose (CMC) + Tween 80.

Table II. – *In vivo* activity of the DFMO derivatives on the *T. b. brucei* CMP/mouse model.

Loiseau *et al.*, 1997a; Deverre *et al.*, 1989). The active principle is released from the lipidomimetic ligand by hydrolysis. Such a strategy is valuable for compounds having rapid elimination from plasma compartment because it allows to maintain therapeutic concentrations of the active principle in plasma. Thus, previous studies showed that the presence of an ester bond between palmityl groups and glycerol allowed the release of the active principle by the action of esterases (Loiseau *et al.*, 1994). The purpose is here to obtain derivatives stable in intestinal medium to be cleaved slowly in plasma. The replacement of ester bounds by ether or amide bounds is responsible for a delayed effect of hydrolysis (Elkihel *et al.*, 1994). Thus, O-DFMO and S-DFMO were designed as DFMO lipidomimetic derivatives able to resist intestinal esterases. Nevertheless, they were also not cleaved in plasma and act themselves by a specific mechanism of action which remains to be investigated. The most important result from this study is the finding of a compound (O-DFMO) having *in vitro* trypanocidal properties and a slight *in vivo* effect. It was not possible to give higher doses because of suspension volume too high for a treatment by oral route in mice. Further studies could be developed on drug formulation in order to have suspension volume allowing treatments using higher doses.

ACKNOWLEDGEMENTS

Dr C.D. Houldworth, Research Support Manager at Marion Merrell Dow Research Institute (Strasbourg, France) is greatly acknowledged for supplying Eflornithine.

REFERENCES

- BACCHI C.J., NATHAN H.C., YARLETT N., GOLDBERG B., MCCANN P.P., SJOERDSEMA A. *et al.* Combination chemotherapy of drug-resistant *Trypanosoma brucei rhodesiense* infections in mice using DL- α -difluoromethylornithine and standard trypanocides. *Antimicrobial Agents and Chemotherapy*, 1994, 38, 563-569.
- BAYLES J.D., HARRISON S.M., MBWABI D.L. & SCHECHTER P.J. Treatment of arsenical refractory Rhodesian sleeping sickness in Kenya. *Annals of Tropical Medicine and Parasitology*, 1989, 83, 111-114.
- CLARKSON A.B., BIENEN E.J., BACCHI C.J., MCCANN P.P., NATHAN H.C., HUTNER S.H. *et al.* New drug combination for experimental late-stage African trypanosomiasis: DFMO with suramine. *American Journal of Tropical Medicine and Hygiene*, 1984, 33, 1073-1077.
- DEVERRE J.R., LOISEAU P.M., COUVREUR P., LETOURNEUX Y., GAYRAL P. & BENOIT J.P. *In vitro* evaluation of filaricidal activity of GABA and 1,3-dipalmitoyl-2-(4-aminobutyl)glycerol HCl: a diglyceride prodrug. *Journal of Pharmacology and Pharmacology* 1989, 41, 191-193.
- DOUA F., BOA F.Y., SCHECHTER P.J., MIEZAN T.W., DIAI D., SANON F.R. *et al.* L' α -difluorométhylornithine (Eflornithine) dans le traitement de la trypanosomiase humaine africaine à *T. gambiense* au stade tardif: efficacité et tolérance. *Bulletin de la Société de Pathologie Exotique et de ses Filiales*, 1988, 81, 589-590.
- ELKIHIL L., LOISEAU P.M., BOURASS J., GAYRAL P. & LETOURNEUX Y. Synthesis and orally macrofilaricidal evaluation of niclosamide lymphotropic prodrugs. *Arzneimittel Forschung/Drug Research*, 1994, 44, 1259-1264.
- HAEGELE K.D., ALKEN R.G., GROVE J., SCHECHTER P.J. & KOCHWESER J. Kinetics of DFMO: an irreversible inhibition of ornithine decarboxylase. *Clinical and Pharmacological Therapeutics*, 1981, 30, 210-217.
- JENNINGS F.W. Relative efficacy of melarsen oxide compared with mel Cy (Cymelarsan) when used in combination with difluoromethylornithine in the treatment of trypanosomiasis of the central nervous system. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1992, 86, 257-258.
- KAMINSKY R., GUMM I.D., ZWEYGARTH E. & CHUMA F. A drug incubation infectivity test (DIIT) for assessing resistance in trypanosomes. *Veterinary Parasitology*, 1990, 34, 335-343.
- LEVIN V.A., CSEJTEY J. & BYRD D.J. Brain, CSF, and tumor pharmacokinetics of α -difluoromethylornithine in rats and dogs. *Cancer Chemotherapy and Pharmacology*, 1983, 10, 196-199.
- LOISEAU P.M., WOLF J.G. & GAYRAL P. Spiroarsoranes of second generation: evaluation of trypanocidal properties against *Trypanosoma brucei brucei*. *Tropical Medicine and Parasitology*, 1993, 44, 37-36.
- LOISEAU P.M., DEVERRE J.R., ELKIHIL L., GAYRAL P. & LETOURNEUX Y. Study of lymphotropic targeting and macrofilaricidal activity of a melphalan prodrug on the *Molnema desetae* model. *Journal of Chemotherapy*, 1994, 6, 230-237.
- LOISEAU P.M., BOURASS J. & LETOURNEUX Y. Lymphotropic antifilarial agents derived from closantel and chlorambucil. *International Journal for Parasitology*, 1997a, 27, 443-447.
- LOISEAU P.M., CHAUFFERT O. & CZOK M. Effect of chronic trypanosomiasis on the bioavailability of α -difluoromethylornithine (DFMO) after oral administration: pharmacokinetics study on DFMO plasma levels in infected and noninfected mice using a high-performance liquid chromatography assay. *Parasitology Research*, 1997b, 83, 386-389.
- MOSMANN T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 1983, 65, 55-63.
- SIMARRO P.P. & NDONG ASUMU Gambian trypanosomiasis and synergism between melarsoprol and eflornithine: first case report. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1996, 90, 315.
- VAN NIEUWENHOVE S. Advances in sleeping sickness therapy. *Annales de la Société Belge de Médecine Tropicale*, 1992, 72, 39-51.

Reçu le 11 février 1998
 Accepté le 30 mars 1998