TOXOPLASMA ENCEPHALITIS: INFLUENCE OF THE VEHICLE ON THE EFFICACY OF DIFFERENT DOSES OF 2′,3′-DIDEOXYINOSINE IN MICE

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Summary :

In this study we investigated the effect of the antiretroviral molecule 2′,3′-dideoxyinosine (Videx®) against cerebral cysts in a murine model of toxoplasmic encephalitis caused by a wild cystic strain of *Toxoplasma gondii*. The role of the vehicle was also studied. Three doses were used: 50, 100 and 150 mg/kg of body weight/day. The doses of 50 and 150 mg/kg were prepared by dissolving pure 2′,3′-dideoxyinosine powder in Maalox® suspension before gavaging the mice; the dose of 100 mg/kg was prepared by grinding tablets of Videx® that were suspended in water. A decrease in the number of cysts and a morphological modification of them were noted from day 15 with the lowest dose. The most important decrease could be observed with the dose of 100 mg/kg/d. After 30 days of treatment with this dose, 65 % of the cysts were destroyed compared to controls. For the doses of 50 and 150 mg/kg/d prepared with Maalox®, 36 % and 51 % of the cysts were destroyed respectively. So ddI has an effect on the cerebral cysts of *T. gondii* even at a low dose. The galenic formulation influences its action since the doses prepared with Maalox® were less efficient than those prepared from ground tablets.

KEY WORDS : *Toxoplasma gondii*, toxoplasmosis, 2′,3′-dideoxyinosine, mice, vehicle.

Résumé : ENCÉPHALITE TOXOPLASMIQUE : INFLUENCE DE L'EXCIPIENT SUR L'EFFICACITÉ DE DIFFÉRENTES DOSES DE 2',3'-DIDÉOXYINOSINE CHEZ LA SOURIS

Dans cette étude nous avons étudié l'efficacité de la molécule antirétrovirale 2′,3′-didéoxyinosine (Videx®) contre les kystes cérébraux dans un modèle murin de toxoplasmose cérébrale due à une souche kystogène sauvage de Toxoplasma gondii. *L'importance de l'excipient a été également étudié.*
Trois doses ont été utilisées : 50, 100 et 150 mg/kg de poids corporel/jour ont été préparées. Pour les doses 50 et 150 mg/kg, la poudre pure de 2′,3′-didéoxyinosine a été mise en suspension dans du Maalox® avant de l'administrer aux souris; la dose de 100 mg/kg/j a été préparée en broyant des comprimés de Videx® et mise en suspension dans de l'eau. Une diminution du nombre des kystes ainsi qu'une modification morphologique de ceux-ci ont été observées dès la plus faible dose. La plus importante diminution a été observée pour la dose de 100 mg/kg/j. Au bout de 30 jours de traitement à cette dose, 65 % des kystes ont été détruits par rapport au groupe témoin. Pour les doses de 50 et 150 mg/kg/j préparées avec du Maalox®, respectivement 36 % et 51 % des kystes ont été détruits. La ddI exerce donc un effet sur les kystes cérébraux même à faible dose. La formulation galénique influence son action puisque les doses préparées avec le Maalox® ont été moins efficaces que celles préparées avec les comprimés broyés.

MOTS CLÉS : Toxoplasma gondii, *toxoplasmose, 2′,3′-didéoxyinosine, souris, excipient.*

Although the frequency of toxoplasmic encephalitis has been greatly reduced by a better control of the HIV thanks to the new therapeutic strategies, this protozoan disease remains a significant concern in failing or immunocompromised patients (Richards *et al.*, 1995; Leport *et al.*, 1996; Klepser (Klepser, 1997). Today, many molecules have been tested *in vitro* and *in vivo* whether alone or in association. Many of these molecules come from antimicrobial research. The group of macrolides includes molecules such as clarithromycin (Araujo *et al.*, 1992), azithromycin (Blais *et al.*, 1993a; Wiselka *et al.*, 1996), clindamycin (Blais *et al.*, 1993b). Others molecules have been developed such as ketolides (Araujo *et al.*, 1997) or a fluoronaphtyridone trovafloxacin (Khan *et al.*, 1997). The sulfonamide group includes molecules such as sulfadiazine (Harris *et al.*, 1988), sulfamethoxazole (Carr *et al.*, 1992), dapsone (Derouin *et al.*, 1991; Payen *et al.*, 1997) and the pyrimidine group includes pyrimethamine (De Gans *et al.*, 1992). New ways of treatment have arisen since the demonstration of the anti-*Toxoplasma* activity of atovaquone (Araujo *et al.*, 1991). A new approach for the treatment of *Toxoplasma* encephalitis may consist in an action on the purine salvage pathway of this parasite. 2′,3′-dideoxyinosine (ddI), an inosine analog used in the therapy of HIV+ patients for its antiviral action, has shown an efficacy in animals with a daily dose of 100 mg/kg of body weight for 30 days (Sarciron *et al.*,

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1997). In the present report, we investigated the effect of different doses of ddI on the number of cerebral cysts in treated mice. ddI was given in conjunction with an antiacid buffer in order to increase the drug bioavailability.

MATERIALS AND METHODS

MICE

Fifty female NMRI mice, eight weeks-old, weighing 30 g at the beginning of the experiment were used in the study. They were given water and food *ad libitum* throughout the experiments.

PARASITES

Tachyzoites of the cyst-forming strain (strain DUR) of *T. gondii* were isolated from the amniotic fluid of a pregnant woman. This avirulent strain was maintained in our laboratory by oral passage of cysts from the brain of an infected mice. Each mouse was infected orally with 10 tissue cysts.

DRUG AND TREATMENT

2',3' dideoxyinosine, as bulk ddI powder and commercial Videx® tablets containing active principle and vehicle were obtained from Bristol-Myers-Squibb France. Pure ddI was suspended in liquid Maalox® (suspension of aluminium and magnesium hydroxydes). The Videx® tablets were suspended in water after grinding. The experiments were performed with pure ddI at the doses of 50 and 150 mg/kg of body weight, and Videx® commercial tablets at the dose of 100 mg/kg of body weight. Each concentration was administered orally, by gavage as a single daily dose. Each mouse received the appropriate dose under a volume of 0.2 ml. The treatment of infected mice was initiated three-months after infection. In both cases, treatment was given for 30 days. Infected mice were separated in five groups of 10 mice each: group 1 (controls) mice received only water; group 2 mice were treated with Maalox®; groups 3 and 4 mice were treated with 50 or 150 mg/kg of body weight respectively and group 5 mice were gavaged with tablets at the daily dose of 100 mg/kg of body weight. Each group of animals was subdivised in three. For the first sub-group, the mice were examined after 15 days of treatment, the second after 30 days of treatment and for the last sub-group, the mice were sacrificed 30 days after the last dose. The animals were euthanasied one week after the end of treatment. The brain of each mouse was taken out for cysts count, microscopical and histological studies as described by Sarciron *et al.*, 1997.

STATISTICAL ANALYSIS

The significance of differences was evaluated by Student's t test.
P values ≤ 0.001 were considered significant.

RESULTS

No mice died during the experiments whatever the dose used. Table I presents the data on the number of cerebral cysts from the control mice during the different times of the study. After 15 days of treatment, a decrease of about 20 % non significant ($P > 0.001$) in the number of cysts in treated mice at 50 and 150 mg/kg of body weight compared with controls was observed. A decline of about 50 % ($P < 0.001$) of cerebral cysts was noted in mice treated at the daily dose of 100 mg/kg of body weight. After 30 days of cure, a decrease of 36 % and 51 % was observed at the doses of 50 or 150 mg/kg of body weight respectively, whereas a decrease of 65 % was noted with 100 mg/kg of body weight.
Thirty days after the end of treatment, a decrease of 49 % and 63 % for 50 and 150 mg/kg of body weight and 68 % in animals treated by a dose of 100 mg/kg of body weight were found. The microscopical study of cerebral cysts revealed that the cysts in control mice, untreated or gavaged with Maalox®, had an identical morphology which remained unchanged during all the time of the experiments (data not shown). In the mice treated for 15 days with 50 mg/kg of body weight, the cysts had an apparently normal wall and contained less bradyzoites than the controls. After 30 days of cure with this dose, no bradyzoite was visible although the

Mice group (treatment regimen)	**Number of cysts/ml**		
Duration of treatment	15 days	30 days	+ 30 days
Group 1 (Controls)	287 ± 15	350 ± 18	370 ± 15
Group 2 (Maalox® control group)	300 ± 20 NS***	333 ± 21 NS***	352 ± 18 NS***
Group 3 (ddI* 50 mg/kg)	233 ± 12 NS***	217 ± 14 (p<0.001)***	183 ± 18 (p<0.001)***
Group 4 (ddI* 150 mg/kg)	233 ± 20 NS***	167 ± 15 (p<0.001)***	133 ± 11 (p<0.001)***
Group 5 (Videx®** 100 mg/kg/d)	150 ± 11 (p<0.001)***	116 ± 5 (p<0.001)***	116 ± 7 (p<0.001)***

* pure ddI powder was suspended in Maalox® suspension.
** Videx® tablets were suspended in water.
*** Versus group 1.
NS : non significant.

Table 1. – Number of mice cerebral cysts per ml (± standard deviation) after 2',3'-dideoxyinosine treatment per os of 15 days, 30 days and 30 days after the last dose of the 30 days treatment.

wall of the cyst seemed intact. A month after the end of the treatment, brain tissue cysts were morphologically unchanged.

After 15 days of treatment with a dose of 150 mg/kg of body weight, the bradyzoites were hardly distinguishable while the cystic wall seemed to remain intact. After 30 days, the cysts had an ovalar aspect and contained a small number of bradyzoites. This morphological aspect was found again in the treated cysts one month after the end of treatment.

The mice treated for 15 days at the daily dose of 100 mg/kg of body weight showed cerebral cysts with an irregular form and which seemed devoid of bradyzoites. After 30 days of treatment, the cysts still remaining in the cerebral tissue had always an irregular form with an optically empty interior. A month after the end of the treatment, observation and identification of the cysts were difficult to make because of their very altered morphological aspect and the absence of bradyzoites.

DISCUSSION

This study confirms our previous results which had evidenced a reduction of about 70 % in the number of cerebral cysts after 30 days of treatment at the daily dose of 100 mg/kg of body weight (Sarciron *et al.*, 1997). A dose two fold lower (50 mg/kg of body weight) was effective on the number of cysts in the cerebral tissue. Of great importance are the effects of the doses used and their relationship to the vehicle employed. Pure ddI powder suspended in an aqueous mixture of aluminium and magnesium hydroxyde was used, as well as ground commercial tablets suspended in water. Calcium carbonate and magnesium hydroxyde were used in the formulation of tablet vehicle. These compounds are anti-acids and avoid degradation of the acid labile N-glycosidic bond of 2',3'-dideoxyinosine (Perry & Balfour, 1996). A decrease in the number of cysts whatever the dose used and from 15 days of treatment was noted, with the most important decline observed with the dose of 100 mg/kg of body weight. This latter result seemed to be related to the galenic formulation. So, we suppose that either the ground commercial tablet is more resistant to the murine gastric acidity than the pure powder suspended in Maalox®, or Maalox® leads to a reduced absorption of ddI.

The morphological damage to the cysts was greater and occurred more rapidly with the 100 mg/kg/d dose compared to the one of 150 mg/kg/d. One month after the end of treatment, and whatever the dose used, no cyst with a morphological aspect comparable to controls could be seen. The absence of bradyzoites was certainly due to the non viability of cysts. The effect of ddI seemed irreversible because the retroinfestations realised from treated mice at the daily dose of 100 mg/kg of body weight for one month were negative in our previous experience (Sarciron *et al.*, 1997). Beginning at 15 days of treatment, a modification of its aspect was observed, leading to its fragilisation. According to our hypothesis, this fragilisation induced an increase of the wall permeability triggering a rapid alteration of bradyzoites. The bradyzoites were eventually damaged by ddI or its metabolites, since ddI is active against tachyzoites *in vitro* (Sarciron *et al.*, 1998).

Whatever the initial presentation of the drug before dissolution: pure powder or tablets, an effect on cerebral cysts of *T. gondii* has been shown, even at a low dose. The same efficiency was obtained with 300 mg/kg/d clarithromycin during eight weeks or 200 mg/kg/d clarithromycin-minocycline combination plus 50 mg/kg/d during four weeks (Araujo *et al.*, 1992). A study on the target of this molecule on the cyst wall is under way. To our knowledge, a small number of drugs target the cyst wall. The mode of action of drugs against *T. gondii* is often unknown, and the understanding of their mechanism of action comes from bacteria models (Araujo *et al.*, 1997). As the majority of these drugs targets protein synthesis, the mechanism of action of ddI may be similar. ddI inhibition of HIV reverse transcriptase argue towards this hypothesis. Nevertheless, the possibility for ddI to inhibit membrane protein synthesis cannot be excluded.

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