Effects of Multiple Doses of Isoprinosine on Echinococcus multilocularis Metacestodes

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Isoprinosine was given at daily doses of 0.5, 1, 2, and 4 g kg−1 of body weight to jirds that were infected for 3 months with Echinococcus multilocularis metacestodes. The effects of the different drug doses on metacestodes were studied by transmission electron microscopy and biochemical methods. At lower doses, increases in uric acid and adenosine deaminase activity were noted. At 4 g kg−1 of body weight, marked ultrastructural damage with metabolic perturbations was observed.

In recent years, benzimidazoles and their derivatives have been introduced for the treatment of alveolar echinococcosis and hydatid disease (5, 18). The first compound used was mebendazole, which causes an irreversible inhibition of glucose uptake, the main energy source for most members of the subclass Cestoda (1, 8, 17). More recently, albendazole is preferred and is considered to have efficacy against alveolar and hydatid disease equal to that of mebendazole (4, 7, 19). However, in addition to its serious adverse effects such as hepatotoxicity, neutropenia, or alopecia (13), albendazole therapy is not sufficient to induce the death of Echinococcus multilocularis metacestodes, but it does lead to a somewhat delayed course of infection. In fact, treated Echinococcus granulosus and E. multilocularis metacestodes from gerbils are able to redevelop after a latency period (14, 15). Moreover, albendazole is known for its action on the germinal layer of E. granulosus, and its efficacy is known to be inversely proportional to the development of the laminated layer (9, 10); as a consequence, this drug does not act when the parasite is well developed. For all these reasons, we began to study another metabolic pathway. Exploration of metabolic pathways of parasites should provide the necessary knowledge of the range of therapeutic molecules and possible alternatives for the treatment of those infections. The purpose of this report is to outline briefly the effect of a drug that is commonly used for its immunostimulatory effect and that is also thought to act on the purine pathway. The purine pathway, in fact, seems to be an important target for chemotherapy against parasites (11, 12). Isoprinosine (from Delalande Laboratory) is a complex formed from inosine, p-acetamidobenzoic acid salt, and N-dimethylamino-2-propanol. The effects of different doses of isoprinosine given to jirds that were infected 3 months previously with E. multilocularis metacestodes were studied. We found that the drug affected the structure and metabolism of the larvae, and we studied these effects by transmission electron microscopy and biochemical methods.

Fifty milligrams of E. multilocularis metacestodes taken from a stock infection in jirds (Meriones unguiculatus) was implanted in the abdominal cavities of 3-month-old jirds. At 90 days postinfection the animals were divided into five groups.

The first group (5 animals) received a daily oral dose of isoprinosine of 0.5 g kg of body weight−1, the second group (5 animals) received a daily oral dose of 1 g kg−1, the third group (5 animals) received a daily oral dose of 2 g kg−1, and the fourth group (15 animals) received a daily oral dose of 4 g kg−1. The fifth group (15 animals) was made up of untreated infected animals.

Isoprinosine was presented in powder form, 650 mg of which was equivalent to 500 mg of active principle. The dose per animal was given in a volume of 0.2 ml of water containing 1% arabic gum. Animals were treated during 5 consecutive days, with an additional dose given on day 13 to restimulate the host immune defense system, and the animals were then killed by cervical dislocation on day 19. Metacestodes were collected, cleared of the host tissue, and washed several times in 0.9% sodium chloride (6). Proteins were estimated as described by Bensadoun and Weinstein (2) by using serum bovine albumin as a standard. Glucose was determined by using a gluco-kit Biomerieux 61301 with ortho-toluidine.

The uric acid dosage was based on the following chemical determination. After deproteinization of uric acid by phosphotungstic reduction in an alkaline medium, a color reaction developed and the optical density was determined by spectrophotometry at a λ of 700 nm. The enzymatic activity of adenosine deaminase was determined by the method described by Blake and Berman (3). Control samples contained only 20 mM Tris-HCl (pH 8) and 0.25 M sucrose. For the transmission electron microscopic study, metacestode material from each experiment was collected and treated as described previously (6).

Two animals died during the experiment, one in the third group and one in the fourth group. The weights of the E. multilocularis metacestodes from treated and untreated animals were compared. The mean weight of larvae from controls was found to be 10.48 ± 0.67 g. The mean weight of metacestodes from treated jirds decreased with graded doses of isoprinosine; doses of 0.5, 1, 2, and 4 g kg−1 resulted in mean weights of 8.55 ± 4.83, 5.30 ± 1.09, 5.03 ± 0.31, and 6.07 ± 0.99 g, respectively. A general modification of the macroscopic structure of the treated metacestodes at the different doses of isoprinosine was observed. The treated metacestodes were less multivesicular than controls and had a yellowish appearance.

Biochemical effects of isoprinosine treatment on E. multilocularis metacestodes were also observed. Metacestodes treated with doses of 2 and 4 g kg−1 showed a decrease of

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proteins compared with levels in controls: 4.24 ± 0.04, 4.34 ± 0.15, and 6.34 ± 0.04 mg ml⁻¹, respectively. The glucose concentration of the treated larval mass was increased at doses of 1, 2, and 4 g kg⁻¹ (0.38 ± 0.003, 0.42 ± 0.003, and 0.37 ± 0.002 mmol g of fresh tissue⁻¹), respectively, compared with controls (0.24 ± 0.002 mmol g of fresh tissue⁻¹). A great augmentation of uric acid at isoprinosine doses of 0.5, 1, and 2 kg of body weight⁻¹ was observed in comparison with that in controls: 149.39 ± 0.03, 162.9 ± 0.02, 229.81 ± 0.04, and 8.12 ± 0.03 μM g of fresh tissue⁻¹, respectively. On the other hand, a decrease in the uric acid concentration at an isoprinosine dose of 4 g kg⁻¹ was noted (54.74 ± 0.05 μM g of fresh tissue⁻¹). Under the action of isoprinosine, the enzymatic activity of adenosine deaminase was increased, except for that at the dose of 4 g kg⁻¹ (181.59 ± 0.09 nmol min⁻¹ mg of proteins⁻²), for which the activity was comparable to that of controls (196.10 ± 0.05 nmol min⁻¹ mg of proteins⁻²). Daily isoprinosine doses of 0.5, 1, and 2 kg kg⁻¹ lead to an augmentation of the uric acid accumulation in relation to an increase in specific adenosine deaminase activity. Compared with the structure of untreated metacestodes (Fig. 1), the intensity of the ultrastructural alterations induced by isoprinosine, which are illustrated in Fig. 2, 3, and 4, is closely related to the doses of the drug. Indeed, with the lowest dose, metacestodes showed only moderate damage, consisting of an increased vesiculation of the superficial tegumentary syncytium and parenchyma. With a daily dose of 1 g kg⁻¹, many cellular elements, namely, tegumentary cytons and muscular cells, began to lyse, showing disrupted cytoplasmic membranes and free mitochondria in the interstitial matrix. After a dose of 2 g kg⁻¹, cellular elements disappeared, the superficial tegumentary syncytium was disrupted, and mitochondria, which were preserved or altered, were still visible in the hypervascularized parenchyma. A marked alteration of the metacestodes was obtained with the highest daily dose of isoprinosine (4 g kg⁻¹). Below the laminated layer, all tegumental substructures completely disappeared and were replaced by a loose matrix containing large vacuoles. This important degeneration of metacestodes was confirmed by the reinforcement of mice. Indeed, the mice reinfected with metacestodes treated with 0.4 g of isoprinosine kg⁻¹ did not develop any lesions. Actually, no data are available on the use of isoprinosine in parasitic disease or on the mechanisms of action of this drug. Touraine et al. (16) examined the in vitro and in vivo effects of isoprinosine on T-cell suppressor activity and have demonstrated that this drug is able to act on purine metabolism. In the present study, isoprinosine probably enhanced host cell-mediated functions but also affected the metabolism of the purine pathway in E. multilocularis metacestodes, leading to uric acid accumulation, which could have modified the internal pH of the parasite. In a previous study (6), we have shown important ultrastructural modifications of the parasite associated with the inhibition of the enzymatic activity of lactate dehydrogenase, and we have suggested that E. multilocularis metacestodes are unable to cope with an acidic medium. In this study, the uric acid accumulation in the metacestodes could have been at the origin of the ultrastructural damages; the ultrastructural repercussions of isoprinosine treatment against E. multilocularis were more important than those observed previously with isatin. These results are very promising because of the important damage to E. multilocularis metacestodes caused by a drug that is nontoxic to humans given as a short-term treatment. This work represents a preliminary study. Further study is necessary to elucidate the proposed mechanism.

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REFERENCES


