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BIOCHEMISTRY OF SCHISTOSOMIASIS MANSONI
IV — EFFECTS OF OXAMNIQUINE ON THE HEPATIC LYSOSOMAL ACTIVITY

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SUMMARY

The typical hepatic lesion observed in schistosomiasis mansoni is an inflammatory process that develops around the living or dead worms or around the eggs. This inflammatory response is linked with a greater lability of the lysosomal membranes. Oxamniquine, a drug extensively used in the treatment of this disease, was tested **in vitro** and **in vivo** in order to investigate its effects on the physicochemistry of the lysosomal membranes. The **in vitro** results indicated that there was a stabilizing effect on 27.1% of these particles. The **in vivo** experiments were carried out after the intraperitoneal injection of the drug in a dose of 7.5 mg/kg, and the animals were killed 1, 3, 6, 9 and 12 days after the treatment. The results showed a labilization of 22.7% of the lysosomal membranes after the first day, increasing to 60.1% around the third day. The labilization decreased to 37.2% on the sixth day. On the ninth day, an opposite stabilizing effect was observed in 35.7% of the lysosomes.

INTRODUCTION

The lysosomes are intracellular organellae that contain several hydrolytic enzymes (DINGLE [5]) and are directly or indirectly connected with processes of cellular defense such as cellular endocytosis, digestion, microautophagy, excretion and detoxication.

Many investigators such as SMITH [20]; HIRSCHHORN & WEISSMANN [8]; BONNER et al. [2]; RICCIUTI [16]; STRAUSS [21] and RAO et al. [15] have demonstrated the important role played by lysosomes as mediators of the inflammatory response and cellular lysis. PANAGIDES [11] has shown that these particles contain several proteins which can induce inflammation. Besides many acute inflammatory diseases, many others of genetic origin characterized by intracellular accumulation of non-metabolised molecules, some of which evolve with a chronic inflammatory process, have been described as resulting from one or more lysosomal enzymatic failures (HIRSCHHORN & WEISSMANN [8]).

The present most accepted explanation for the outbreak of the inflammatory process seems to be centered in the destruction of the lysosomal membranes or other less radical physicochemical changes. These changes are almost always followed by the liberation of several hydrolytic enzymes able to initiate the inflammatory response especially at the cellular level, being capable, depending on the degree of cellular lysis, of extending it secondarily to the tissue level.

Schistosomiasis mansoni, a parasitosis widely disseminated in our environment, is cha-

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racterized by an inflammatory process which develops around the living or dead worms and/or the eggs. According to RODRIGUES & SOARES [17], the lysosomes, especially those of the liver, exhibit an increased lability of their membranes directly proportional to the degree of liver lesion caused by that parasitosis.

Oxamniquine (6-hydroximethyl-2-isopropylaminomethyl-7-nitro-1, 2, 3, 4-tetrahydroquinoline) is extensively employed in the treatment of schistosomiasis mansoni, orally and parentally (CLARKE et al.[3]; COUTINHO et al.[4]; SILVA et al.[19]; EYAKUSE[6]; FOSTER[7]; KATZ et al.[9]; PEDRO et al.[12], PELLEGRINO et al.[13] and PRATA et al.[14]). In spite of extensive clinical studies of its toxic effects **in vivo** — it is classified as a substance of questionable toxicity by CLARKE et al.[3]; COUTINHO et al.[4]; KATZ et al.[9]; PRATA et al.[14]; RODRIGUES et al.[18] demonstrated that this drug caused the most varied effects **in vitro** on the activity of several seric enzymes originating for the most part in the lysosomes.

As a result of these observations, we decided to study the **in vitro** and **in vivo** effects of oxamniquine on isolated mice liver lysosomes treated and not treated with this substance.

MATERIALS AND METHODS

Adult albino mice of either sex, normaly fed, were separated into three groups. The control group consisted of 20 mice; the group for **in vitro** experiments also contained 20 mice and the third group, for t he **in vivo** observations, contained 100 mice.

This last group received one intraperitoneal injection of oxamniquine in the dose of 7.5 mg/kg, and was sacrified in lots of 20 mice 1, 3, 6, 9 and 12 days after the treatment.

The animals were killed without anesthetic. Their livers were immediately separated from the gallbladder and cut into small pieces in a buffered solution at pH 7.4, refrigerated at 2.°C. This solution was composed of: saccharose 25.10^{-2} M, dissodic phosphate 5.10^{-3} M, monopotassic phosphate 5.10^{-3} M, potassium chloride 10^{-2} M, tris (hydroximethyl) aminomethane-TRIS 10^{-2} M and ethylenediaminetetra acetic acid-EDTA 2.10^{-4} M. After two washings in order to take out most of the blood, the pieces were resuspended in the proportion of one part of tissue to ten of the same solution. Then this suspension was homogenized in the apparatus of Potter-Elvejhem with 250 r.p.m. for a maximum of 2 min. in an ice-bath. The lysosomal fraction was isolated by fractionated centrifugation and refrigerated at 2°C, according to the outline in Fig. 1.

The **in vitro** action of oxamniquine on the lysosomal activity was estimated, after incubation of the lysosomes of each liver, isolated in sediment III in an ice-bath for 20 minutes, with 225 μg of the drug. That dose was calculated from the one used **in vivo**, that is, 7.5 mg/kg.

For the **in vivo** experiments, in which the animals had been previously treated with intraperitoneal injections of oxamniquine, the lysosomes isolated in sediment III were simply incubated during 20 minutes in the buffered solution, in the same conditions as the controls. After incubation, the intact lysosomes were separated by new centrifugation at 32,000 g for 20 min. in sediment IV. The acid phosphatase activity, (orthophosphoric monoesterphosphohydrolase 3.1.3.2), measured according to the technique of ANDESCH & SZCYPINSKI[1] in supernatant IV, reflects the physicochemical conditions of stability of the lysosomal membranes.

The total proteins of each experiment were evaluated according with the technique of LOWRY et al.[10].

RESULTS

The standard lysosomal activity obtained from lysosomes isolated from livers of non-treated animals and not submitted to the **in vitro** action of oxamniquine, in terms of acid phosphatase, was 11.56 ± 2.55 mU/mg of total proteins. In Fig. 2 can be seen the stabilizing effect brought about **in vitro** by oxamniquine on the lysosomal membranes.

The activity of the acid phosphatase in these lysosomes when treated **in vitro** has been 8.42 ± 2.42 mU/mg and this represents a protective lysosomal effect of 27.1% in relation to the control lysosomes. Statistical analysis of these results showed a $t^o = 5$, which is highly significant at the level of 1%. Figure 3 shows the results of the **in vivo** experiments. In them

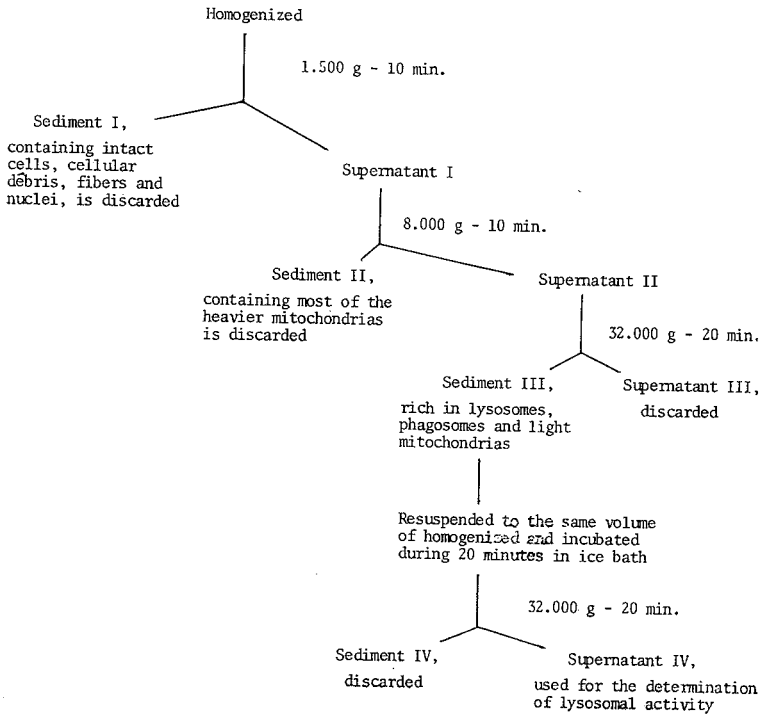

Fig. 1

IN VITRO PROTECTIVE EFFECT OF OXAMNIQUINE ON LYSOSOMAL MEMBRANES

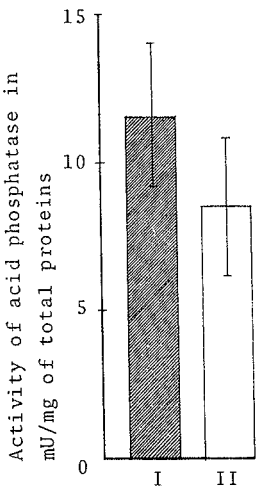

Fig. 2

I - Control lysosomal activity.

II - *In vitro* treated lysosomes with oxamniquine.

the lysosomes were obtained from livers of animals previously treated with oxamniquine and isolated after 1, 3, 6, 9 and 12 days. The lysosomal activities for these samples were as follows: 1 day after the oxamniquine treatment — 14.18 ± 4.42 mU/mg; after 3 days — 18.51 ± 3.49 mU/mg; 6 days — 15.86 ± 3.11 mU/mg; 9 days, 7.44 ± 1.08 mU/mg, and finally, after

IN VIVO EFFECTS OF OXAMNIQUINE ON LYSOSOMAL MEMBRANE

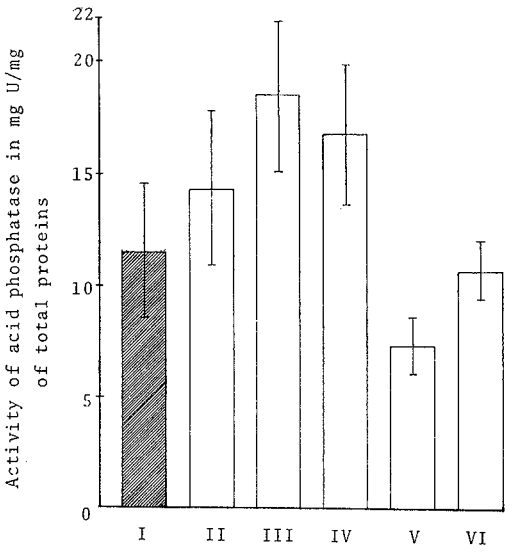

Fig. 3

I - Control lysosomal activity.

II - Lysosomal activity 1 day after treatment.

III - 3 days, IV - 6 days, V - 9 days, VI - 12 days after treatment *in vivo* with oxamniquine.

12 days — 10.72 ± 1.16 mU/mg. Compared with the lysosomal activity control, these values showed an increasing lability of the lysosomal membranes till the third day, decreasing till the ninth day, when, conversely, a stabilizing effect corresponding to 33.15% was observed, with return to normality by the twelfth day.

DISCUSSION

The **in vitro** protecting effect exerted by oxamniquine on the lysosomal membranes in shown in Fig. 2. It was fast, corresponding to 27.1%. This means that more than one fourth of treated lysosomes became more stable and resistant to the lysis normally observed in the course of the different steps of the experimentation. The quickness of the effect seems to be linked to the direct contact of oxamniquine in solution with these cellular particles and this makes possible the interaction between that substance and the lysosomal membranes.

The physicochemical behavior of the membranes of lysosomes isolated from the livers of animals treated with oxamniquine is shown in Fig. 3. This substance worked practically like a labilizing agent till the sixth day after its application. The effect diminished in intensity between the sixth and the ninth days. Later on it even became stabilizing agent, losing at this point its activity on these particles, which then behaved physicochemically like the control particles. The intraperitoneal injection of oxamniquine labilized 22.7% of the lysosomes after the first day. This effect was even greater, i.e., 60.1%, around the third day. On the sixth day the labilization decreased to 36,2%. On the ninth day, an opposite stabilizing effect on 35.7% of the lysosomes was observed. The protecting effect **in vivo** was significantly greater than that **in vitro.**

The duplicity of the effects observed in the **in vivo** experiments may be explained by the fact that oxamniquine, after having been endocyted by the liver cells, starts reactions that lead to the labilization of lysosomes, particles responsible for the cytoplasmic detoxication processes. After the sixth day, it is possible that the drug is incorporated by these particles,

thus promoting its effect of stabilization on their membranes in the same way as **in vitro.** The detoxication of oxamniquine should justify the return to the physicochemical normality of the membranes.

Finally, the **in vitro** and **in vivo** stabilizing effects caused by oxamniquine on lysosomal membranes allow an extrapolation to its clini cal use as one of the therapeutic agents of schistosomiasis mansoni, because it will probably not contribute to the aggravation of those inflammatory reactions of liver parenchyma so characteristic of this parasitosis.

RESUMO

Bioquímica da esquistossomose mansônica. IV — Efeitos da oxamniquine sobre a atividade lisossomial hepática

Uma das características mais importantes e que sempre acompanha a lesão hepática causada pela esquistossomose mansônica, é o processo inflamatório que se desenvolve em torno do verme vivo ou morto, assim como do ovo deste parasita. Este processo inflamatório está intimamente ligado à uma maior labilidade das membranas lisossomiais. A oxamniquine, droga extensivamente usada no tratamento desta doença, foi testada **in vitro** e **in vivo** para investigação de possível efeito físico-químico sobre as membranas dos lisossomas. Os resultados **in vitro**, indicaram que esta droga, possui efeito estabilizador sobre 27,1% destas partículas intracelulares. As observações **in vivo**, foram levadas a efeito, após a injeção intraperitonial da droga, na dose de 7,5 mg/kg de peso, em camundongos. Estes animais, assim tratados, foram utilizados, 1, 3, 6, 9 e 12 dias após o tratamento. Os resultados, mostraram uma labilização de 22,7% das membranas lisossomiais, após o primeiro dia, aumentando para 60,1% em torno do terceiro dia. No sexto dia o tratamento, o efeito labilizador decresceu para 37,2%. Já no nono dia ,observou-se o oposto, um efeito estabilizador na ordem de 35,7% dos lisossomas. Após o décimo segundo dia, estas organelas voltaram à normalidade físico-química de suas membranas.

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