Biochemical Pharmacology, Vol. 27, pp. 473–474. Pergamon Press, 1978. Printed in Great Britain.

# BIOCHEMICAL STUDIES ON β-GLUCURONIDASE ENZYME IN LIVER AND SPLEEN HOMOGENATES OF NORMAL AND *SCHISTOSOMA MANSONI* INFECTED MICE TREATED WITH HYCANTHONE

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(*Received* 19 *May* 1977; *accepted* 10 *August* 1977)

**Abstract**—Hycanthone methanesulphonate (the active metabolite of lucanthone) was administered to normal and to *Schistosoma mansoni* infected mice. In both cases, the β-glucuronidase enzyme activity was found elevated in the whole tissue homogenates of the liver and spleen. The rise in activity was progressive and prolonged. We could conclude that hycanthone is a hepatotoxic drug and is possibly carcinogenic in mice.

An increased β-glucuronidase activity has been reported in the urine of patients suffering from urinary schistosomiasis [1–3]. A marked drop in the enzyme activity was recorded after administration of schistosomicidal drugs [2] so that Fripp (1966) [3] suggested the determination of β-glucuronidase activity level as a test of cure for urinary bilharziasis.

In a previous work in our laboratory Saad *et al.* [4], noted a raised β-glucuronidase activity in the whole tissue homogenates of liver and spleen of mice infected with *Schistosoma mansoni*. The present work aimed at studying the effect of the schistosomicidal drug hycanthone (1-{[2-(diethylamino) ethyl] amino } - 4 - ( hydroxymethyl ) - thioxanthen - 9 - one ) (Etrenol, Winthrop Product Inc., New York, U.S.A.) in modifying the enzyme activity levels in these two organs. Normal and *Schistosoma mansoni* infected animals were compared with their corresponding controls.

## MATERIALS AND METHODS

Swiss albino mice, 2 months of age and 15 g of weight, were divided into four groups: Group I: served as normal controls. Group II: served to study the effect of hycanthone. Group III: served as *S. mansoni* infected controls. Group IV: served to study the effects of both *S. mansoni* and hycanthone. Mice in groups III and IV were infected by the paddling technique [5] with 100 *S. mansoni* cercariae. Ten days later, mice in groups II and IV were given a dose of hycanthone. A second injection was administered after 30 days. Hycanthone was injected intramuscularly in a dose of 30 mg/kgm body wt. Mice from all groups were sacrificed 20, 40, 60, 90 and 120 days from the date of infection. Each time, 4–6 mice were autopsied by exsanguination after stunning by a blow on the head. A sample of the liver was proceeded for histopathological examination.

*Preparation of homogenates.* The fresh liver and spleen were quickly removed and placed in ice cold bidistilled water. In crude water suspensions, the enzyme was soluble and fully active at the usual tissue concentrations employed for β-glucuronidase assay with phenolphthalein glucuronide [6, 7]. Tissue homogenates (0.6 g in 25 ml water [7], based on the wet wt of the tissue) were prepared by using a Potter–Elvehjem homogenizer.

*Determination of β-D-glucuronidase.* The method of Talalay *et al.* [8] was used with slight modification. The reaction mixtures (0.1 ml substrate (0.01 M), 0.2 ml. 2.4 per cent whole tissue homogenates and 0.7 ml. McIlvain buffer pH 5.0) were incubated in test tubes in a shaking water bath at 37° with air as the gas phase, for 2 hr. During this period, the velocity of hydrolysis was constant and linearity maintained [8].

## RESULTS

The activity of β-glucuronidase enzyme was expressed in units. One unit liberated 1 μg phenolphthalein per hour at 37° [8]. The values obtained from normal mice and the other groups were compared statistically using student's *t*-test. Differences with probability values less than 0.05 were considered significant [9].

Results of the changes in β-glucuronidase activity in the liver of the different groups by time are presented in Table 1. They demonstrate that the administration of hycanthone was followed by a raised β-glucuronidase activity which was constantly significant, late after injection (60 days after infection). Histopathological findings in the liver confirmed these results. In the two groups receiving hycanthone an intense cellular infiltration together with fatty change in the parenchymal cells were observed which became more pronounced on the fourth month after treatment. Some cells were abnormally large, with hyperchromatic nuclei some of which showed abnormal division (Fig. 1).

In the spleen, the enzyme activity of normal control mice had a mean value of 3263 units/g/hr and a S.D. of 194. After hycanthone administration, a pro-

Table 1. β-glucuronidase activity* in whole tissue homogenates of liver of the different mice groups by duration of infection

| Mice group | Days after infection 20 | 40 | 60 | 90 | 120 |
|---|---|---|---|---|---|
| Normal control | | | | | |
| No. of mice | | | 12† | | |
| Mean | | | 2291 | | |
| S.D. | | | 131 | | |
| Normal treated | | | | | |
| No. of mice | 6 | 6 | 6 | 6 | 6 |
| Mean | 2812 | 3299 | 3351 | 3698 | 5129 |
| S.D. | 66 | 169 | 191 | 277 | 113 |
| Infected | | | | | |
| No. of mice | 4 | 4 | 4 | 4 | 4 |
| Mean | 2240 | 2326 | 2621 | 4323 | 3919 |
| S.D. | 180 | 126 | 273 | 138 | 155 |
| Infected treated | | | | | |
| No. of mice | 6 | 6 | 6 | 6 | 6 |
| Mean | 2422 | 2569 | 3021 | 4336 | 4635 |
| S.D. | 138 | 236 | 95 | 337 | 300 |

* Results are expressed in β-glucuronidase units/g tissue/hr.
† Mice were of different ages.

gressive rise in activity was observed with figures as high as 4597 and a S.D. 145 in the infected treated mice.

## DISCUSSION

*Schistosoma mansoni* infection in the mouse was reported to be accompanied by an elevation in the β-glucuronidase enzyme activity in the whole liver and spleen homogenates [4]. In the present work, the schistosomicidal drug hycanthone was administered to *S. mansoni* infected mice. The enzyme activities in both liver and spleen tissue homogenates did not decrease, but it increased parallel to the level in the infected control mice and even superseded it late in infection. Similarly when the drug was injected to non-infected mice, it incited an increase in the β-glucuronidase activity which was particularly pronounced 4 months from the date of infection.

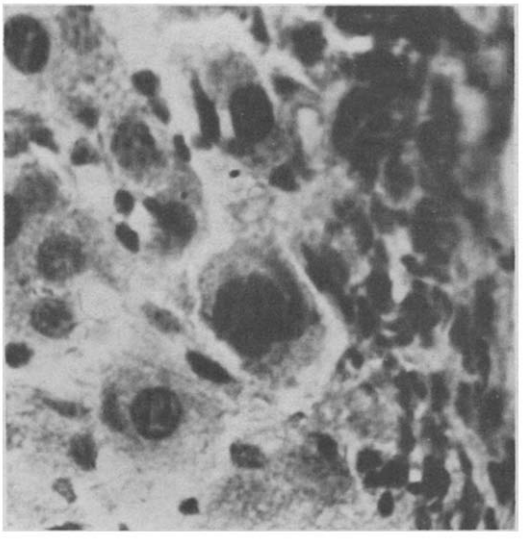

Fig. 1. Hycanthone treated bilharzial mouse liver. Note the cellular infiltration and the hyperchromatic nuclei. ×820.

Hycanthone methanesulphonate was reported to be a hepatotoxic drug [10]. Hepatotoxic agents were reported to increase the β-glucuronidase enzyme in the liver [11]. The increased β-glucuronidase activity observed in the present study adds more evidence to the hepatotoxicity of the drug. Hepatic cirrhosis is accompanied with an increased oestrogen hormone level in the blood [12]. Oestrogen has a striking stimulating effect on the reticulo-endothelial system [13]. Since the liver and spleen contain the highest concentration of reticulo-endothelial cells, the result is hyperplasia and enlargement of these organs. The administration of hycanthone to mice was described to be accompanied with a lymphocytic cellular infiltration [14]. Lymphocytes as well as reticulo-endothelial cells are rich in lysosomes and in β-glucuronidase enzyme [15]. This may explain the rise in hepatic and splenic β-glucuronidase.

The increase in β-glucuronidase enzyme was progressive and of long duration after cessation of drug administration. This may point to the possible carcinogenicity of hycanthone in the mouse. Histopathological findings confirmed this possibility. It is well known that β-glucuronidase enzyme increases with malignancy [11, 16]. Hycanthone has been described to show mutagenic and teratogenic potentials [17, 18]. Haese *et al.* [19] observed hyperplasia and hepatomas in mice infected with *S. mansoni* and treated with hycanthone.

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