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IN VITRO EFFECTS OF SOME ANTIMONIAL AND NON-ANTIMONIAL SCHISTOSOMICIDAL DRUGS ON LIVER β-GLUCURONIDASE ENZYME ACTIVITY

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Abstract—The effect of some antimonial and non-antimonial drugs on liver β-glucuronidase activity was studied *in vitro*. The antimonial drugs Tartar emetic, Astiban, Stibophen and Bilharcid increased β-glucuronidase activity at the highest concentration tested. Anthiomaline was the only drug which produced a significant increase in the enzyme activity at all the concentrations used. Stibophen and Anthiomaline showed a positive correlation between their concentrations and their activation effects on the enzyme activity. The non-antimonial drugs Etrenol and Oxamniquine induced a pronounced inhibitory effect at the higher drug concentrations tested. Etrenol exhibited a negative correlation between its concentration and the activity of liver β-glucuronidase. The results revelead that these schistosomicidal drugs could exert their activation or inhibition on the liver β-glucuronidase activity, at least in part, by direct action on the enzyme.

In Egypt, the association between vesical schistosomiasis and bladder cancer is generally accepted, but the exact nature of this association is still not clear [1]. One of the factors that could account for the biochemical aspects of bilharzial bladder cancer is the abnormality in amounts of urinary carcinogenic tryptophan metabolites [2]. The carcinogenic compounds appear to be released in the bladder from their conjugated glucuronides through the action of β-glucuronidase [3]. Elevated urinary activities of β-glucuronidase were reported in patients with *Schistosoma haematobium* infestation [4, 5] and in bladder cancer patients [6, 7]. This led some investigators to suggest that the β-glucuronidase enzyme may be a predisposing factor in the genesis of bladder carcinoma [3].

Recently, our studies revealed that the activity of liver β-glucuronidase was increased in *Schistosoma mansoni*-infected mice treated with Etrenol [8], Oxamniquine [9] and Astiban [10]. However, these *in vivo* studies have not clarified whether the increase in the enzyme activity was a result of the direct effect of these drugs on liver β-glucuronidase or due to the *de novo* synthesis of the enzyme protein.

It was planned, therefore, to study the *in vitro* effects on the activity of liver β-glucuronidase of some antimonial and non-antimonial schistosomicidal drugs that are commonly used in Egypt and elsewhere.

MATERIALS AND METHODS

Bovine liver β-glucuronidase and its substrate phenolphthalein monoglucuronide were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. The antimonial drugs used, were potassium antimony tartarate (Tartar emetic, Cid Co., Egypt), lithium antimony mercaptosuccinate (Anthiomaline, Spicia, Paris), sodium antimony dimercaptosuccinate (Astiban, Roche, Switzerland), pentasodium antimony bischatecol-3,5-disulphonate (Stibophen, Alexandria Co., Egypt) and piperazine diantimonyl tartarate (Bilharcid, Cid Co., Egypt). All the above drugs contained trivalent antimony.

The non-antimonial drugs were methanesulphonate derivative of hycanthone; 1-{[(2-(diethylamino) ethyl] amino}-4-(hydroxymethyl)—thioxathen—9—one (Etrenol, Winthrop, U.S.A.) and 6-hydroxymethyl-2-isopropylaminomethyl-7-nitro-1, 2, 3, 4-tetrahydroquinolone (Oxaminique, Pfizer, U.S.A.).

The activity of liver β-glucuronidase was determined using the method of Talaly *et al.* [11]. The incubation medium contained 0.1 mM phenolphthalein β-glucuronide, 100 Fishman units of liver β-glucuronidase and different concentrations of the schistosomicidal drugs in a final volume of 1 ml of 0.1 M acetate buffer, pH 4.5. The reaction was started by the addition of the substrate and the tubes were incubated at 37° for 1 hr. The reaction was terminated by the addition of 4 ml 0.4 M glycine buffer, pH 10.5. Blanks were obtained in the same way but the enzyme was added after the addition of glycine buffer. The absorption readings of the clear supernatant fluid were measured at 550 nm. The enzyme activity was expressed in Fishman units [11]. One enzyme unit is equivalent to 1 μg phenolphthalein released per hour at 37° under the above experimental conditions. The results of the present study were statistically evaluated using the Student *t*-test and correlation coefficient analysis [12].

RESULTS

Table 1 summarizes the *in vitro* effects of some commonly used antimonial and non-antimonial schistosomicidal drugs on the activity of liver β-glu-

Table 1. Effect of some schistosomicidal drugs on the activity of liver β-glucuronidase enzyme*

Schistosomicidal drug	β-Glucuronidase activity ± S.D.				
	Molar drug concentration (mM)				
	10^{-3}	10^{-2}	0.1	1.0	10.0
Control†	(26.12 ± 0.77)				
Tartar emetic	26.0 ± 1.47	25.8 ± 0.89	26.7 ± 1.02	26.3 ± 0.7	34.8 ± 0.22
Anthiomaline	31.2 ± 0.46	31.3 ± 0.47	33.3 ± 0.88	35.5 ± 1.05	38.5 ± 0.87
Bilharcid	26.2 ± 0.73	26.8 ± 0.25	25.3 ± 1.67	25.5 ± 1.50	33.5 ± 1.04
Astiban	26.5 ± 1.05	25.8 ± 1.07	26.8 ± 2.08	28.8 ± 1.25	32.7 ± 1.19
Stilbophen	22.5 ± 1.80	23.7 ± 1.17	24.2 ± 1.01	26.8 ± 0.67	35.5 ± 1.36
Etrenol	28.8 ± 1.43	25.2 ± 1.48	21.8 ± 1.69	18.7 ± 1.10	12.7 ± 0.82
Oxamniquine	35.9 ± 1.12	31.3 ± 1.01	26.5 ± 1.02	22.86 ± 0.75	20.5 ± 0.76

* The activity of liver β-glucuranidase enzyme is expressed in Fishman units. These values represent the mean ± S.D. of ten experiments carried out for each drug.

† The incubation medium contained all materials for enzymatic assay and the drug solvent. Each drug has a control experiment. The control value represents the mean ± S.D. of β-urea control experiments. The mean value of the different control experiments showed no significant difference in enzyme activity in the presence of different drug solvents.

curonidase. Tartar emetic, Astiban and Bilharcid did not affect the β-glucuronidase activity at the low drug concentrations. They induced significant activation only at the highest drug concentration tested (10 mM). The percentage activation was 33.2 per cent ($P < 0.001$), 21.4 per cent ($P < 0.005$) and 28.3 per cent ($P < 0.001$), respectively.

Anthiomaline activated β-glucuronidase enzyme at the lower drug concentrations and this effect was increased by increasing the drug concentration. The percentage activation increased from 19.3 per cent ($P < 0.01$) at 10^{-3} mM up to 47.4 per cent ($P < 0.001$) at 10 mM. The statistical analysis confirmed the existance of a positive correlation between Anthiomaline concentration and the activation effect on β-glucuronidase enzyme ($r = 0.87$, $P < 0.05$).

Although Stibophen induced inhibitory effect on liver β-glucuronidase activity at the low drug concentrations tested, this effect was statistically insignificant ($P > 0.05$). Stibophen significantly stimulated ($P < 0.001$) the activity of liver β-glucuronidase only at 10 mM. Also, there was a positive correlation between the increase in the activity of liver β-glucuronidase and the range of Stibophen concentrations studied ($r = 0.98$, $P < 0.001$).

On the other hand, at 10^{-3} mM the methanesulphonate derivative of hycanthone, Etrenol, induced a slight activation effect (10.4 per cent $P > 0.05$) on the liver β-glucuronidase enzyme. When the drug concentration was increased gradually up to 10 mM, the enzyme activity was decreased simultaneously to 51.5 per cent inhibition at the highest drug concentration tested. The inhibitory effect of Etrenol on liver β-glucuronidase was markedly observed at 0.1 mM ($P < 0.05$), 1.0 mM ($P < 0.001$) and 10 mM ($P < 0.001$). Moreover, a statistically significant negative correlation existed between the activity of the enzyme and the drug concentrations tested ($r = 0.84$, $P < 0.05$). Oxamniquine exhibited an activation effect on the activity of liver β-glucuronidase at the lower drug concentrations 10^{-3} mM and 10^{-2} mM, where the percentage activation was 37.5 per cent ($P < 0.001$) and 19.95 per cent ($P < 0.005$), respectively. At the higher drug concentrations tested, 1 and 10 mM Oxaminiquine induced inhibitory effects. The percentage inhibition was 12.48 per cent ($P < 0.05$) and 21.52 per cent ($P < 0.001$), respectively. The statistical analysis revealed insignificant correlation between Oxamniquine concentration and its inhibitory effect on the liver β-glucuronidase enzyme activity ($r = 0.67$, $P > 0.05$).

DISCUSSION

The results of the present study revealed that the antimonial drugs Tartar emetic, Astiban and Stibophen have no significant effect on the activity of liver β-glucuronidase at the low concentrations tested, from 10^{-3} to 1 mM. However, these antimonial drugs induced significant activation of enzyme activity at the highest concentration tested (10 mM). Anthiomaline was the only antimonial drug which exhibited a significant activation of β-glucuronidase enzyme at all the drug concentrations tested. It is interesting to notice that the two drugs, Stibophen and Anthiomaline, which showed a positive correlation between their stimulatory effect on the enzyme activity and the range of the drug concentrations tested, have low antimony content. They contain 13.5 and 16 per cent antimony, respectively, whereas Astiban, Tartar emetic and Bilharcid have 25, 36.5 and 37.0 per cent antimony, respectively [13]. It seems, therefore, that the schistosomicidal drugs with low-antimony content show a statistically significant relationship between concentration and effect on liver β-glucuronidase enzyme activity.

The antimonial drugs may be one of the important factors which cannot be ignored as affecting β-glucuronidase enzyme activity. It was found that the enzyme activity increased in the urine of bilharzial patients treated with Tartar emetic [14]. Serum β-glucuronidase activity significantly increased after treatment of bilharzial patients with Astiban, while urinary enzyme activity was found to be unaffected [15]. Moreover, increased β-glucuronidase enzyme activity has been reported in whole liver tissue homogenates of non-infected and *Schistosoma mansoni-*

infected mice treated with Astiban [10]. The present study shows that Tartar emetic and Astiban directly stimulated the activity of liver β-glucuronidase enzyme only at the highest drug concentration tested (10 mM).

On the other hand, the non-antimonial drugs Etrenol and Oxamniquine induced pronounced inhibitory effects on the activity of the liver β-glucuronidase enzyme at the higher drug concentrations tested (1 and 10 mM). However, Oxamniquine induced a marked activation effect on β-glucuronidase enzyme at the lower drug concentrations tested. Etrenol exhibited a significant negative correlation between the drug concentrations and the activity of liver β-glucuronidase enzyme, whereas this relationship was not observed in the case of Oxamniquine. This may reflect the dependence of the enzyme activity on the concentration of Etrenol, which was reported to be a hepatotoxic drug [16]. Etrenol, as compared with Oxamniquine, seems to be a more effective inhibitor of the activity of liver β-glucuronidase enzyme. Etrenol, at 10 mM, induced 51.5 per cent inhibition, whereas Oxamniquine exhibited 21.5 per cent inhibition at the same molar concentration.

Our previous studies have revealed that administration of Etrenol to normal and *Schistosoma mansoni*-infected mice increased the activity of β-glucuronidase enzyme in whole tissue homogenates of liver and spleen [8] and of bladder [17]. Fripp has reported that urinary β-glucuronidase of bilharzial patients falls to the normal range a few days after treatment with Etrenol and sodium antimony tartrate [18]. Oxamniquine has no apparent effect on serum and urinary β-glucuronidase of bilharzial patients [19], whereas administration of this drug to normal and *Schistosoma mansoni*-infected mice was accompanied with elevation in liver β-glucuronidase enzyme activity [9].

From the results already described, it is clear that the antimonial and non-antimonial drugs could, at least in part, exert their activation or inhibition on liver β-glucuronidase activity by direct action on the enzyme. These effects may account for the high incidence of bladder tumours observed in bilharzial patients repeatedly treated with antimony-containing drugs.

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