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SHORT COMMUNICATIONS

Hepatic drug conjugation/deconjugation systems in hepatosplenic schistosomiasis

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Hepatosplenic schistosomiasis is a parasitic liver disease resulting in granuloma formation followed by liver fibrosis. Studies of the mixed function oxidase system in experimental hepatosplenic schistosomiasis suggested that the activity of the system was impaired [1, 2]. Isolation of the hepatocyte from the granulomata revealed that this enzyme system was moderately reduced in the acute phase of the disease (8 weeks) with a return to normal by 14 weeks [3]. Although phase II drug metabolism is as important as phase I in biotransformation of xenobiotics and endogenous compounds, little attention has been given to the drug conjugation system in hepatosplenic schistosomiasis.

The present study was designed to provide data on hepatic conjugation/deconjugation enzyme systems in schistosomiasis isolated from associated hemodynamic changes which can hinder drug delivery.

Methods

Animal preparation. Male Balb/c mice were exposed to 100 *Schistosoma mansoni* cercariae (Egyptian strain) by subcutaneous injection at the age of 5 weeks. A second group of unexposed mice was used as control. Animals were killed in batches by cervical dislocation at 10, 14 and 20 weeks post exposure. After being killed, 0.5 g of liver was set aside for the study of enzyme activities in total liver homogenates. The remainder of the liver was used for the isolation of parenchymal and granulomatous tissues and preparation of their respective homogenates.

Separation of hepatic parenchyma and granuloma. Representative liver samples from infected mice 14 weeks post exposure were used for the isolation of hepatic parenchyma and granuloma [4]. After homogenization of the liver in a Waring blender, the granuloma remained intact because of its firmer consistency and was retained on a nylon mesh (110 μm). The collected granuloma were washed several times by repeated sedimentation. The filtrate was saved as a source for the parenchymal homogenate. The collected fractions and the total liver samples were then homogenized in a Polytron homogenizer and assayed for specific transferase and hydrolase activities.

Analytical procedures. Methylumbelliferone in addition to its glucuronide and sulfate conjugates were employed as substrates to measure activities of transferases and hydrolases associated with glucuronide and sulfate conjugation/deconjugation [5].

Glutathione *S*-transferase activity was assayed using 1-chloro-2,4-dinitrobenzene as a substrate [6]. These assays were carried out under conditions where the reaction rate was linear with protein concentration and time.

Results and Discussion

The transferases involved in glucuronide, sulfate and glutathione conjugation were assayed in total liver homogenates (Table 1) at three different times post-infection with *S. mansoni* representing early, intermediate and late chronic stages of the infection. Both glucuronosyl trans-

Table 1. Hepatic transferase activities in total liver homogenates and their distribution during experimental hepatosplenic schistosomiasis

Post-infection/ preparation	nmol/mg protein/hr UDP-glucuronosyl transferase		nmol/mg protein/hr Sulfotransferase		nmol/mg protein/hr Glutathione *S*-transferase × 10^{-3}	
	C	Inf	C	Inf	C	Inf
10 Weeks						
T. Hgte	25.6 ± 1.5 (11)	19.3 ± 4.1† (11)	52.8 ± 7.3 (9)	84.2 ± 10.9† (9)	6.5 ± 1.5 (10)	4.1 ± 0.9+ (10)
14 Weeks						
T. Hgte	30.3 ± 2.7 (10)	23.6 ± 5.0† (10)	67.8 ± 9.0 (11)	64.6 ± 8.5 (11)	6.8 ± 1.3 (9)	5.0 ± 1.2* (9)
Par. Hgte	—	30.6 ± 7.4	—	97.4 ± 15.1	—	8.0 ± 2.4
Gran. Hgte	—	11.0 ± 3.1‡	—	14.7 ± 4.6‡	—	0.7 ± 0.4‡
20 Weeks						
T. Hgte	36.6 ± 13.0 (10)	23.8 ± 5.1* (10)	65.3 ± 18.4 (10)	74.3 ± 18.8 (12)	8.5 ± 2.5 (11)	5.5 ± 1.3† (11)

Values are means ± SD of transferase activities determined in total liver homogenates (T. Hgte) from control (C) and infected (Inf) mice (100 *Schistosoma mansoni* cercariae) as described in Methods. In addition, activities of parenchymal (Par) and granulomatous (Gran) homogenates were assessed at 14 weeks. Data were analysed statistically using Student's *t*-test.

The number in parentheses represents number of animals assayed.

* $P < 0.01$ (compared to control).

† $P < 0.001$ (compared to control).

‡ $P < 0.001$ (compared to parenchymal activities).

Table 2. Activities of hepatic hydrolases in total liver homogenates and their distribution during experimental hepatosplenic schistosomiasis

Post-infection/ preparation	nmol/mg protein/hr β-Glucuronidase C	β-Glucuronidase Inf	Sulfatase C	Sulfatase Inf
10 Weeks				
T. Hgte	33.0 ± 4.1 (11)	55.0 ± 5.9* (11)	9.1 ± 1.7 (11)	9.1 ± 1.0 (11)
14 Weeks				
T. Hgte	35.2 ± 4.1 (10)	54.9 ± 8.9* (10)	7.9 ± 1.9 (10)	11.7 ± 2.1* (10)
Par. Hgte	—	64.7 ± 12.6	—	11.4 ± 3.1
Gran. Hgte	—	74.2 ± 11.1	—	7.0 ± 1.5†
20 Weeks				
T. Hgte	38.6 ± 9.0 (7)	56.4 ± 8.1* (7)	12.1 ± 4.1 (8)	20.1 ± 2.8* (8)

Values are means ± SD.

Enzyme activities were measured in liver homogenates (T. Hgte) from control (C) and infected (Inf) mice as described in Methods. In addition, the distribution of these hydrolases between the parenchyma (Par) and granuloma (Gran) at 14 weeks was determined. Data were analysed statistically by Student's *t*-test.

Number in parentheses, represents number of assayed livers.

* $P < 0.001$ (compared to control).

† $P < 0.01$ (compared to parenchymal activity).

ferase and glutathione transferase activities were reduced at all stages of infection. A reduction in glutathione *S*-transferase activity could result in increased exposure of the liver to toxic reactive intermediates with a higher incidence of toxicity and carcinogenicity [7, 8]. In contrast, sulfotransferase activity was significantly increased in infected livers at 10 weeks post infection (159% of control activity, $P < 0.001$) with a return to the control value at 14 weeks. This selective increase of sulfotransferase activity during the acute phase of the infection could be attributed, at least in part, to the stresses incurred as a result of egg deposition and early hepatic inflammation which result in stimulation of endogenous steroid production [9].

The distribution of transferase activities between the parenchymal and granulomatous tissue was determined in the schistosomal liver at 14 weeks following infection (Table 1). Glucuronyl transferase activity in the parenchymal homogenate was approximately 3 times the activity in the granulomatous homogenate ($P < 0.001$). The other two transferases assayed in this study, sulfotransferase and glutathione *S*-transferase were primarily localized in the parenchymal tissue with only 15 and 9% of parenchymal activities being detected in the granuloma, respectively.

Table 2 shows the hydrolase activities in total liver homogenates at 10, 14 and 20 weeks post infection. Both hydrolases examined, β-glucuronidase and arylsulfatase, increased markedly following infection. These two hydrolases were differently distributed in the schistosomal liver at 14 weeks post exposure. β-Glucuronidase activity was evenly distributed between the parenchyma and the granuloma, while arylsulfatase activity in the granulomatous tissue was more than 60% of the parenchymal activity. Since these two hydrolases are present in lysosomes in addition to microsomes, their increased activities could be explained by the marked accumulation of lysosome rich macrophages at the site of hepatic egg deposition. This observation was also made by Higuchi *et al.* [10], who found a good correlation between an increased arylsulfatase activities and the size of the granuloma. In our study, sulfotransferase activity was significantly increased during the acute phase of infection with no change in sulfatase activity. Accordingly, the sulfation of an increasing number of compounds such as *N*-hydroxyacetylaminofluorene could lead to more toxic conjugates which cause cell necrosis or chemical carcinogenesis [11]. With progress in infection, the sulfation pathway was altered favoring the desulfation of xenobiotics and endobiotics such as estrogens. This may, at least in part, play a role in the development of feminizing characters in male patients with advanced hepatosplenic schistosomiasis [12].

In summary, the biotransformation capacity of the liver is altered in hepatosplenic schistosomiasis being more in favor of deconjugation pathways. These alterations should be considered when xenobiotics are administered and in studies of hepatic toxicity and carcinogenicity.

Pharmacology Department
Faculty of Medicine
Assiut University and
Biochemistry Division
U.S. Naval Medical Research Unit 3
Cairo, Egypt

MOHAMED EL MOUELHI
MOUSTAFA M. MANSOUR

Address for reprints: Research Publication Branch, NAMRU-3, FPO, New York 09527-1600, U.S.A.

REFERENCES

1. Ghazal A, Mahfouz A, Makar A and Ghoneim M, Effect of schistosomal infection and of schistosomicidal drugs on the drug metabolizing enzymes of mouse liver. *Naunyn Schmiedebergs Arch Pharmacol* **282**: 181–186, 1974.
2. Cha Y and Edwards R, Effect of *Schistosoma mansoni* infection on the hepatic drug metabolizing capacity of mice. *J Pharmacol Exp Ther* **199**: 432–440, 1976.
3. El Mouelhi M, Black M and Phillips SM, Hepatic cytochrome P-450 system in experimental hepatosplenic schistosomiasis: presence of an artifact in spectrophotometric analysis. *Biochem Pharmacol* **36**: 2621–2626, 1986.
4. Pellegrino J and Brener Z, Method for isolating schistosome granulomas from mouse liver. *J Parasitol* **42**: 546, 1956.
5. El Mouelhi M, Didolkar M, Elias EG, Guengerich

Fand and Kauffman F. Hepatic drug-metabolizing enzymes in primary and secondary tumors of human liver. *Cancer Res* **47**: 460–466, 1987.
6. Habig W, Pabst M and Jakoby W. Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* **249**: 7130–7139, 1974.
7. Degen G and Neumann H. The major metabolite of aflatoxin B, in the rat is a glutathione conjugate. *Chem Biol Interact* **22**: 239–255, 1978.
8. Orrenius S and Jones D. Functions of glutathione in drug metabolism. In: *Functions of Glutathione in Liver and Kidney* (Eds. Sies H and Wendel A), pp. 164–175. Springer, Berlin, 1978.
9. Thompson T, Watkins J, Gregus Z and Klaassen C. Effect of microsomal enzyme inducers on the soluble enzymes of hepatic phase II biotransformation. *Toxicol Appl Pharmacol* **66**: 400–408, 1982.
10. Higuchi M, Ito Y, Fukuyama K and Epstein W. Biochemical characterization of arylsulfatases detected in granulomatous inflammation. *Exp Mol Pathol* **40**: 70–78, 1984.
11. Mulder G, Meerman J and Vanden Goorbergh A. Bioactivation of xenobiotics by conjugation. In: *Xenobiotic Conjugation Chemistry* (Eds. Paulsen G, Caldwell J, Hutson D and Menn J), p. 282. American Chemical Society, 1986.
12. Kew M, Kirschner M, Abrahams G and Katz M. Mechanism of feminization in primary liver cancer. *New Engl J Med* **296**: 1084–1088, 1977.

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Characterization of repair inhibition by methotrexate of ethylmethanesulfonate- and ultraviolet irradiation-induced DNA damage in Chinese hamster cells*

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As an inhibitor of dihydrofolate reductase (DHFR†), thymidylate synthase (TS), and aminoimadazolecarboxamide-phosphoribosyl transformylase (AICAR), methotrexate (MTX) interferes with the synthesis of adenylate, guanylate, and thymidylate [1]. Through depletion of nucleotide pools, MTX causes inhibition of semiconservative DNA synthesis. Recently, we demonstrated that treatment of cells with MTX also leads to inhibition of DNA synthesis required for the repair of ethylmethanesulfonate (EMS)- or UV-induced DNA damage [2]. MTX was compared to a drug combination known to block the DNA synthesis step of excision repair, hydroxyurea + Ara-C (H/A). Although MTX was found to inhibit the repair of both types of damage, its efficacy was equivalent to H/A after EMS-but not UV-induced damage. These data suggested that MTX was more efficacious at inhibiting the short patch mode of repair. The ability of MTX to affect repair was prevented by coadministration of hypoxanthine (HX) and thymidine (TdR), indicating that MTX-induced nucleotide depletion was responsible for repair inhibition.

To further understand the mechanism by which MTX inhibits these forms of DNA repair, we have studied the dependency of EMS repair inhibition on both pretreatment time and concentration of MTX. The basis for the selectivity with which MTX inhibited EMS repair more effectively than UV repair has also been examined. We found that MTX in combination with Ara-C was as effective as H/A in causing inhibition of UV repair.

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† Abbreviations: DHFR, dihydrofolate reductase; AICAR, aminoimidazolecarboxamide-phosphoribosyl transformylase; MTX, methotrexate; SSB, single strand breaks; HU, hydroxyurea; Ara-C, cytosine arabinofuranoside; H/A, HU + Ara-C; EMS, ethylmethanesulfonate; UV, ultraviolet irradiation; CHO, Chinese hamster ovary; REB, rad equivalent breaks; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; TS, thymidylate synthase; HX, hypoxanthine; and TdR, thymidine.

Methods

Tissue culture reagents were obtained from Gibco (Grand Island, NY). Tissue culture flasks were obtained from Corning (Corning, NY) and tissue culture dishes from Costar (Cambridge, MA). Unless otherwise indicated, all other reagents were obtained from Sigma (St. Louis, MO).

Cell culture and drug exposure conditions. The AA8 CHO cell line used in this work was grown in monolayer in DMEM supplemented with dialyzed calf serum (5%, v/v), 100 units/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37°. In all experiments, cells were allowed to recover for at least 8 hr after plating before drug treatment was commenced.

For EMS repair experiments, 600,000 cells were plated into 25 cm^2 flasks, 8 hr later MTX was added, and the cells were incubated for an additional 16 hr. Exposure to EMS was carried out by first transferring 5 of the 10 mL of medium present in each treatment flask to sterile tubes; the transferred medium, which were kept at 37°, was used to refeed the cells after treatment with EMS. EMS was then added to the cells to achieve a final concentration of 1.8 mg/mL. One hour later, the drug-containing medium was replaced with the medium removed earlier. For UV repair experiments, 600,000 cells were plated into 10-cm dishes. Eight hours later, MTX was added to the cells. Sixteen hours later (immediately prior to irradiation), the medium was transferred to sterile tubes and was used to refeed the cells immediately after exposure to 10 J/m^2 of 254 nm UV light delivered from a germicidal lamp. For both UV and EMS repair experiments, repair was terminated by replacing the medium with ice-cold PBS and keeping the flasks or dishes on ice until harvesting the cells for alkaline elution analysis.

Assay of excision repair by alkaline elution. The time-course of excision repair was monitored by using alkaline elution to measure SSB at various time points following the induction of DNA damage. This technique to follow repair is based on the fact that the excision of damaged regions creates single-strand breaks (SSB) in the DNA, whereas the filling of gaps and their ligation to the parent DNA serves to remove the breaks. In the presence of inhibitors of DNA repair synthesis, such as H/A, a large accumulation of SSB results due to the build up of excision gaps along the DNA [3–5]. The alkaline elution procedure used in