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# Hepatic drug-metabolizing enzymes in lung tumor-bearing rats

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Drug metabolism, which is subject to modification by many physiological factors [1], may also be altered in many pathological situations. A number of reports indicate that hepatic drug metabolism is impaired in tumor-bearing animals [2–5]. This impairment has been shown to be associated with decreased levels of hepatic cytochrome P-450 and $b_5$, and NADPH cytochrome *c* reductase [5–7]. It has also been reported that hepatic microsomal enzymes obtained from tumor-bearing animals metabolize several drugs at a lower rate than liver microsomal enzymes prepared from normal rats [3, 8]. A relevant study in this line shows increased serum antipyrine half-life in patients with hepatic malignancies [9]. These changes have been attributed to the defective metabolizing capacity of the uninvolved tissue of

liver [9]. Studies available in the literature concern mainly the effects of extrahepatic transplanted tumor cells on hepatic drug metabolism. However, reports on the effect of extrahepatic tumors induced by carcinogenic agents on hepatic drug metabolism are lacking. Therefore, the present study was undertaken to examine the possible influence of lung tumors induced by benzo[*a*]pyrene on the cytochrome P-450-dependent drug-metabolizing enzymes of liver in rats. Also, antipyrine half-life was estimated in tumor-bearing animals so as to evaluate the *in vivo* liver function and to possibly predict the pharmacokinetics of other administered drugs.

*Materials and methods*

Chemicals used were: benzo[*a*]pyrene, cytochrome *c*, uridine diphosphate glucuronic acid (UDPGA), glutathione (GSH), and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. 1-Chloro-2,4-dinitrobenzene (CDNB) was purchased from the Aldrich Chemical Co., Milwaukee, WI, U.S.A. Antipyrine-*N*[*methyl*-$^{14}$C] (sp. act. 50 mCi/mmole) was purchased from Amersham, England. All other chemicals were of reagent grade.

Male Wistar rats weighing 140–160 g were used in these experiments. Lung tumors were induced by the method of Saffiotti *et al.* [10]. Rats were instilled intratracheally (i.t.) with 30 mg of benzo[*a*]pyrene (BP) suspended in 0.15 M NaCl (BP–ferric oxide :: 1:1, w/w) under light ether anesthesia. A total of three i.t. instillations were given per rat, one week apart. Each instillation consisted of 10 mg BP/0.4 ml per rat. Control rats were animals of the same age and weight kept in the same animal room but in separate cages. This group received ferric oxide suspended in 0.15 M NaCl (sterile) in an amount equivalent to that given to rats used for inducing lung tumors. Some rats died unexpectedly during early weeks of treatment and were found to be cannibalized or exhibited tissue autolysis. These rats were not evaluated in the study. Twenty-eight weeks after the last i.t. instillation of BP, rats were used for biochemical estimations of liver function. About 40% of the rats developed lung squamous cell carcinoma.

In-vivo *drug metabolism.* As an indication of *in vivo* drug metabolism, the biological half-life of antipyrine was determined in control and tumor-bearing rats as described earlier [11]. [$^{14}$C]Antipyrine was injected through the penis vein (18 mg/kg, 18 $\mu$Ci/kg) in saline, 1.0 ml/kg. Blood samples ($\simeq$0.2 ml) were collected from rats under light ether anesthesia by occular vein puncture with dilute heparin flushed glass capillaries at 5, 10, 15, 30, 60 min and 2, 3, 4, 6 and 24 hr after dosing. At this point, the hematocrit value for each individual rat was also noted.

*Radioactivity counting.* The blood sample, 0.1 ml, was vortex-mixed with 2.0 ml of dioxane and centrifuged at 2500 *g* for 15 min. One milliliter of clear organic phase was counted in 10 ml of scintillation fluid consisting of 5.0 g 2,5-diphenylopazole (PPO) and 100 mg 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene (POPOP), and 100 g naphthalene in one liter of toluene–dioxane mixture (1:1, v/v). Quenching was determined by external standardization. Under identical extraction procedures, more than 99% of the radioactivity due to antipyrine was found to be extractable.

In vitro *studies.* Rats were killed by exsanguination under light ether anesthesia. Liver was perfused *in situ* through the portal vein with 40–50 ml of cold 0.15 M NaCl. Tissue was quickly excised and rinsed in cold KCl/Tris buffer (150 mM KCl/50 mM Tris–HCl), pH 7.4. Liver was diced with scissors, homogenized in a Potter–Elvehjem homogenizer and diluted to a concentration of approximately 1.0 g wet wt/4.0 ml with KCl/Tris buffer solution. Part of this was used for estimating glutathione by the method of Moron *et al.* [12]. The rest of the homogenate was first centrifuged at 10,000 *g* for 30 min in a cold centrifuge, and then the supernatant fraction was centrifuged at 105,000 *g* for 60 min in a Beckman ultracentrifuge (model L5-50B). The microsomal pellet thus obtained was resuspended in KCl/Tris buffer, pH 7.4.

Protein was determined according to the method of Lowry *et al.* [13], using dried bovine serum albumin as standard. Cytochrome P-450 and $b_5$ were determined by the method of Omura and Sato [14]. The benzo[*a*]pyrene hydroxylase (AHH) activity was determined by the method of Nebert and Gelboin [15], using 3-hydroxybenzo[*a*]-pyrene for standardization (a gift from Prof. J. N. Keith, IIT Research Institute, Chicago, IL, U.S.A.). The uridine diphosphate glucuronosyl transferase (UDP-GT) activity was determined with *p*-nitrophenol as substrate in digitonin-activated microsomes obtained from native microsomal suspensions (16 mg/ml). This was diluted with an equal volume of 1.5% digitonin following the method of Gorski and Kasper [16]. NADPH cytochrome *c*-reductase activity was estimated by the method of Mazel [17], using cytochrome *c* as substrate. Glutathione *S*-transferase (GT) activity in the post 105,000 *g* supernatant fraction was estimated according to the method of Habig *et al.* [18] with 1-chloro-2,4-dinitrobenzene as substrate.

Determination of statistical significance of differences betwen groups was done by using Student's *t*-test.

*Results and discussion*

The BP-exposed group of rats (tumor-bearing or non-bearing) had body weight, liver weight and microsomal protein yield similar to those of the control group, when killed 28 weeks after the last i.t. instillation of BP. However, [$^{14}$C]antipyrine half-life was found to be significantly ($P < 0.001$) higher in tumor-bearing rats when compared to non-tumor-bearing or control rats (Table 1). These results show that many chemical changes in the liver of the host cannot be attributed to the onset of cachexia or to any alteration in the nutritional state that may be associated with the condition of tumor bearing.

Table 2 depicts the effect of lung tumors on the drug-metabolizing enzymes of liver. Most of the components of the mixed-function oxidase (MFO) enzyme system, including cytochromes P-450 and $b_5$, and AHH were found to be impaired in tumor-bearing animals. These observations are in accordance with a number of previous studies [2–5], indicating the effect of extrahepatic transplanted tumors, and also with the findings of Brown *et al.* [6] who reported a decrease in these components in female Wistar rats bearing mammary gland tumors. The decrease in the contents of cytochrome P-450 and $b_5$ may be due to alterations in the activities of key enzymes involved in the regulation of hepatic heme and hemoprotein synthesis and degradation, evidence for which is recently accumulating [5, 19]. We did not find any change in the activity of NADPH cytochrome *c*-reductase. This suggests that the cytochrome P-450 reducing capacity of the microsomal system is not affected because of tumor burden. Our observation is not in agreement with other reports [3–7], indicating impairment in the activity of this enzyme in rats bearing different types of extrahepatic transplanted tumors. This may be due to a difference in methods of assessment of hepatic NADPH cytochrome *c* reductase activity in our study and other reports. However, a possible reason for this discrepancy is not clear. In reference to transplanted tumors [20, 21], it has been suggested that the changes in hepatic microsomal drug metabolism may be mediated by a humoral factor(s) produced by these tumors. Therefore, as we did not find any apparent evidence of metastasis of lung tumor to the liver, a similar mechanism of a humoral factor(s) producing these alterations in hepatic drug metabolism may also be operative in the present situation.

Activities of UDP-GT and GT, two most important enzymes of conjugation reactions of drug metabolism, were found to be decreased in tumor-bearing animals. Also, the content of glutathione that is utilized in the conjugation

Table 1. Effect of lung tumor on the status of rats and the biological half-life of antipyrine*

| | Body wt (g) | Liver wt (g/100 g body wt) | Microsomal protein (mg/g liver) | Biological half-life (min) |
|---|---|---|---|---|
| Control | 280 ± 25 | 4.2 ± 0.6 | 20.4 ± 3.1 | 122 ± 13 |
| Benzo[*a*]pyrene-treated | | | | |
| Non-tumor-bearing | 290 ± 22 | 4.5 ± 0.6 | 18.6 ± 3.1 | 140 ± 20 |
| Tumor-bearing | 272 ± 21 | 4.6 ± 0.7 | 18.2 ± 3.4 | 282 ± 44† |

* Values are given as means ± S.D., N = 5.
† Tumor-bearing group compared to both the control and the non-tumor-bearing group ($P < 0.001$).

Table 2. Effect of lung tumor on hepatic drug-metabolizing enzymes and glutathione content*

| | Cytochrome P-450 | Cytochrome $b_5$ | AHH | NADPH cytochrome *c* reductase | UDP-GT (digitonin-activated) | GT | GSH |
|---|---|---|---|---|---|---|---|
| Control | 1.16 ± 0.10 | 0.76 ± 0.10 | 156 ± 22 | 78 ± 5.6 | 25.4 ± 2.2 | 717 ± 95 | 4.96 ± 0.55 |
| Benzo[*a*]pyrene-treated | | | | | | | |
| Non-tumor-bearing | 1.17 ± 0.11 | 0.79 ± 0.07 | 130 ± 19 | 75 ± 6.3 | 21.7 ± 2.8 | 682 ± 72 | 4.30 ± 0.5 |
| Tumor-bearing | 0.66 ± 0.05†‡ | 0.57 ± 0.07‡§ | 79 ± 11†‡ | 77 ± 4.9 | 14.7 ± 3.6†‖ | 519 ± 53§‖ | 3.32 ± 0.34†‖ |

* Cytochrome P-450 and $b_5$ contents are expressed as nmoles/mg microsomal protein. AHH activity is expressed as pmoles/min/mg microsomal protein. Activities of UDP-GT and NADPH cytochrome *c* reductase are expressed as nmoles/min/mg microsomal protein and GT activity as nmoles/min/mg post 105,000 *g* supernatant fraction. The GSH content is expressed as μmoles/g liver. Values are given as mean ± S.D., N = 6.
† Tumor-bearing group compared to control ($P < 0.001$).
‡ Tumor-bearing group compared to non-tumor-bearing group ($P < 0.001$).
§ Tumor-bearing group compared to control ($P < 0.01$).
‖ Tumor-bearing group compared to non-tumor-bearing group ($P < 0.01$).

reactions was found to be decreased in tumor-bearing animals when compared to non-tumor-bearing or control animals. Therefore, in addition to the impairment in the oxidative process of drug metabolism, decreases in the activities of these enzymes and the content of glutathione could impair the overall biotransformation and elimination of any drug being metabolized through the MFO enzyme system. This is further supported by the observation that the biological half-life of antipyrine was increased significantly in tumor-bearing animals. This condition can well result in prolonged duration of drugs in body circulation.

The findings reported here have definite implications in experimental chemotherapy. Several anti-cancer drugs including cyclophosphamide, procarbazine, methotrexate and mitomycin C are metabolized by liver microsomal enzymes [22–24]. Therefore, the pharmacokinetics and potential toxicity of these agents may be modified by the observed alterations in hepatic drug metabolism in tumor-bearing animals. The ability of tumor to interfere with hepatic drug-metabolizing capacity may be of clinical importance and should be given due consideration in the management of cancer treatment.

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# The metabolism of aflatoxin $B_1$ by human liver

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The rates of NADPH-dependent metabolism by microsomes isolated from two male liver samples ranged between 1.0 and 2.7 nmoles $AFB_1$/min/mg microsomal protein and microsomes from a female liver gave rates of metabolism of 0.63 and 0.73 nmole/min/mg microsomal protein. The major aflatoxin metabolite detected by HPLC was aflatoxin $Q_1$ (approximately 70–90% of the soluble metabolites). Aflatoxin-8,9-dihydrodiol (10–30%) and aflatoxin $M_1$ were also detected as soluble products of microsomal metabolism.

The ability of human post microsomal supernatant to convert the aflatoxin $B_1$-8,9-epoxide to a glutathione conjugate was investigated. There was little evidence for the production of the glutathione conjugate in a system using human cytosol with quail microsomes or in human S9, or reconstituted S9 incubations.

Animal species differ markedly in their susceptibilities to both the acute and chronic toxicity of mycotoxin aflatoxin $B_1$ [1, 2], and there is evidence supporting a metabolic basis for the species differences to aflatoxin $B_1$ induced hepatotoxicity [3–5]. It is possible to estimate the extent of the activation to aflatoxin $B_1$-8,9-epoxide by microsomal metabolism by assaying $AFB_1$ dihydrodiol as its Tris complex on reverse phase HPLC [6]. A major detoxification product has been recently identified as a glutathione conjugate of the epoxide $AFB_1$–GSH [7].* Both activation and deactivation pathways have been investigated [4, 5]. These

* Abbreviations used: $AFB_1$, aflatoxin $B_1$; Tris, Tris-(hydroxymethyl)methylamine; $AFQ_1$, aflatoxin $Q_1$; $AFM_1$, aflatoxin $M_1$; $AFP_1$, aflatoxin $P_1$; AFB–GSH, 8,9-dihydro-8-(*S*-glutathionyl)-9-hydroxy-aflatoxin $B_1$; HPLC, high performance liquid chromatography; GSH, reduced glutathione.