A Preliminary Characterization of Drug-metabolizing Systems in Preneoplastic Nodules from the Livers of Rats Receiving 2-Acetylaminofluorene in Their Diet *

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The development of preneoplastic nodules in the livers of rats which receive 2-acetylaminofluorene in their diet is a highly interesting model system for studying the process of chemical carcinogenesis.[1] Since it is a metabolite(s) of 2-acetylaminofluorene which is believed to be the immediate carcinogen,[2] it is important to monitor the activity of drug-metabolizing systems at different stages of the carcinogenic process. For example, it is possible that a particular pattern of drug-metabolizing activities can render a cell more susceptible to the carcinogenic effects of 2-acetylaminofluorene.

It has been reported that preneoplastic liver nodules contain less cytochrome P-450 and a lower activity of NADPH-cytochrome *c* reductase and aryl hydrocarbon monooxygenase than control and surrounding liver.[3,4] It has also been shown that nodules are relatively resistant to the necrogenic effect of the heptatotoxins CCl_4 and dimethylnitrosoamine compared to surrounding liver.[5] There was no significant difference in the uptake of the dimethylnitrosoamine.

The phase II enzymes epoxide hydrolase and glutathione S-transferase are of key importance in detoxication. It has been shown that epoxide hydrolase activity in preneoplastic liver nodules is greatly elevated compared with control liver.[6] This finding is not easy to explain, since this enzyme is not known to be involved in the metabolism of 2-acetylaminofluorene.

Here we have measured the activity of various drug-metabolizing enzymes in preneoplastic liver nodules and in the livers of rats that have received 2-actylaminofluorene in their diet for a short time. We have also compared these activities in nodules of different sizes.

Experimental. Male Wistar rats were maintained on a diet containing 0.05% 2-acetylaminofluorene according to a schedule designed to produce preneoplastic nodules.[7] One and three weeks after beginning this diet, as well as after the appearance of preneoplastic nodules (18 – 25 weeks), animals were starved for 48 h and then killed. The livers were removed, nodules dissected out, and microsomes prepared according to Ernster *et al.*[8] Cytochrome P-450,[9] NADPH-cytochrome *c* reductase,[10] epoxide hydrolase[11] and glutathione S-transferase using 1-chloro-2,4-dinitrobenzene or 2,6-dichloro-4-nitrobenzene as substrates[12,13] were measured using standard procedures.

In agreement with earlier reports[3,4] we have found that cytochrome P-450 and NADPH-cytochrome *c* reductase activity in nodules were reduced to 31 and 55%, respectively, of control values (Table 1). In contrast we observed that short-term maintenance on pellets containing 2-acetylaminofluorene gave a slight increase in the activity of NADPH-cytochrome *c* reductase (1 and 3

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Table 1. Activities of some drug-metabolizing enzymes in the liver of rats treated with 0.05% 2-acetylaminofluorene in the diet for 1 and 3 weeks and in preneoplastic liver nodules.

Enzyme(s)	Short-term treatment[f] Control	1 week	3 weeks	Nodules
Cytochrome P-450[a]	0.71	0.73	0.51	0.22
NADPH-cytochrome *c* reductase[b]	64.1	92.6	100	35.3
Epoxide hydrolase[c]	4.1	20.4	25.3	21.4
Glutathione *S*-transferase				
with CDNB[d]	0.93	1.51	1.64	5.30
with DCNB[e]	48	92	85	190

[a] nmol/mg microsomal protein. [b] nmol cytochrome *c* reduced/min mg microsomal protein. [c] nmol styrene glycol produced/min mg microsomal protein. [d] μmol 1-chloro-2,4-dinitrobenzene conjugated/min mg cytosolic protein. [e] nmol 1,2-dichloro-4-nitrobenzene conjugated/min mg cytosolic protein. [f] The figures shown are the means of 3 – 5 experiments. The results of these experiments did not differ from one another by more than 10%.

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Table 2. Activities of some drug-metabolizing enzymes in prenoplastic liver nodules of different size.

Parameter	Nodule size[a] small	medium	large			
Nodule weight (g)	–	–	0.50	0.40	0.45	0.60
Cytochrome P-450[a]	0.30	0.22	0.16	0.22	0.15	0.16
NADPH-cytochrome *c* reductase[b]	44	52	39	40	36	39
Epoxide hydrolase[c]	19.4	22.9	10.2	14.7	14.5	18.1
Glutathione *S*-transferase[d]	5.2	4.0	5.5	4.7	5.2	5.9

[a] nmol/mg microsomal protein. [b] nmol cytochrome *c* reduced/min mg microsomal protein. [c] nmol styrene glycol produced/min mg microsomal protein. [d] μmol 1-chloro-2,4-dinitrobenzene conjugated/min mg cytosolic protein. [e] "Small" nodules were 1 – 3 mm in diameter, "medium" 3 – 5 mm in diameter, and "large" greater than 5 mm in diameter. The small and medium-sized nodules were pooled from 2 livers.

weeks, Table 1) and either no change (1 week) or only a slight decrease (3 weeks) in the level of microsomal cytochrome P-450. When 2-acetylaminofluorene was injected intraperitoneally (50 mg/kg body weight in 0.5 ml polyethylene glycol once daily for 5 days) instead of being administered in the diet, a slight increase (46%) in the content of cytohrome P-450 occurred. The same increase was seen when Sprague-Dawley rats were used.[14]

Also in agreement with other reports[6] is our finding that microsomal epoxide hydrolase activity is increased 6 – 7 fold in the nodules, which is likewise the case after short-term exposure (Table 1). In addition cytosolic glutathione S-transferase activity is elevated 4 – 5-fold in the nodules compared with control liver (Table 1). However, this activity is only increased about 50 – 100% after short-term exposure to 2-acetylaminofluorene. As mentioned above, it is difficult to explain these increases in microsomal epoxide hydrolase and cytosolic glutathione S-transferase activities as a result of exposure to 2-acetylaminofluorene, since neither of these enzymes is known to be involved in the metabolism of this carcinogen. One possibility is that glutathione S-transferase B (*i.e.*, ligandin) binds reactive metabolites of 2-acetylaminofluorene.

In preliminary studies together with Docent Bengt Mannervik in our department we have isolated glutathione S-transferase A, B, and C from liver preneoplastic nodules. For all three enzymes the amount of enzyme protein is much greater in the nodules than in control liver. In addition, the specific activity of glutathione S-transferase B from nodules is about twice as high as that of the control enzyme. We are now attempting to explain this difference on the molecular level.

It was also of interest to compare these drug-metabolizing activities in preneoplastic nodules of different sizes. If the changes are either important for the carcinogenic process or characteristic for tumor cells, then they should be seen in nodules of all different sizes. This is indeed the case, even though large nodules demonstrate somewhat larger changes in the components of the cytochrome P-450 system than do small ones. This may reflect their further development, but may on the other hand simply be a consequence of the fact that it is more difficult to dissect the small nodules free from surrounding tissue.

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