# ONCOLOGY

# Activity of Detoxication Enzymes in Rat Hepatoma 27 Transplanted in Different Organs

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Activities of enzymes of active oxygen forms detoxication and phase II xenobiotic metabolism were measured in rat hepatoma 27 cells transplanted to different organs. Activity of phase II xenobiotic metabolizing enzymes was higher in hepatoma cells growing subcutaneously than in those transplanted into the liver, while activity of active oxygen forms detoxication enzymes (except catalase) was higher in cells transplanted into the liver. Benz(a)pyrene induced the enzyme activities in hepatoma growing both subcutaneously and in the liver.

**Key Words:** *hepatoma; reactive oxygen species; enzymes of phase II xenobiotic metabolism; cell microenvironment*

Cell microenvironment plays an important role in the regulation of cell functions. Transplantation of tumors into various organs is a promising approach to *in vitro* investigation of the role of microenvironment. The results obtained with this method showed that some characteristics of the tumor, *e. g.* morphology, invasion capacity, and some biochemical parameters depend on tumor location. For example, colon carcinoma transplanted into granulation tissue acquires "invasive morphotype" different from tumor cells transplanted into normal tissue [5]. In Novikov hepatoma transplanted subcutaneously the activity of deoxycytidylate deaminase is 100 times lower than in the cells of the same hepatoma transplanted intraperitoneally [11]. The level of sulfhydryl groups and glutathione content in leukemia L1210 cells are higher in cells transplanted into the liver than in tumor inoculated intraperitoneally [2]. Published reports about detoxication enzymes in different cells are devoted mainly to investigation of cytochrome P-450. Hepatocytes cultured on glass or plastic for 2-3 days lose their capacity to express cytochrome P-450, while in hepatocytes grown on matrigel the level of cytochrome P-450 remained close to that in the liver for a long time [10]. The content of cytochrome P-450 is higher in Morris hepatomas transplanted into the liver than intramuscularly [12]. Rat hepatoma 27 cells transplanted intramuscularly lack both constitutive and induced cytochrome P-450 isoforms, while the cells of the same tumor transplanted into the liver possess cytochrome P-450-dependent activities, and injection of phenobarbital or benz(a)pyrene induced the respective isoforms of cytochrome P-450 [7].

These data attest to an important role of microenvironment in the function of phase I enzymes of xenobiotic metabolism, but there are no published data on other detoxication enzymes.

We studied the activity of various detoxication enzymes and their induction in hepatoma 27 cells transplanted subcutaneously or into the liver. Two groups of enzymes were studied: phase II enzymes of xeno-

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biotic metabolism (glutathione-S-transferase, NAD(P)H-quinone oxidoreductase, aldehyde dehydrogenase) and enzymes detoxifying active oxygen forms (superoxide dismutase — SOD, catalase, glutathione peroxidase), as well as glutathione reductase, the enzyme regenerating reduced glutathione.

## MATERIALS AND METHODS

In order to rule out the individual differences tumor growth, hepatoma 27 was transplanted subcutaneously and into the liver to the same rat by injecting a tumor fragment into the tissue through a T-piece. The animals were decapitated 2 weeks after transplantation. For enzyme induction the animals were intraperitoneally injected with benz(a)pyrene (30 mg/kg in olive oil) 3 days before sacrifice. Histological studies showed no difference between tumor specimens transplanted subcutaneously or into the liver. Liver and tumor tissue was homogenized in a medium containing 0.25 M sucrose, 50 mM Tris-HCl, 0.05 mM EDTA, and 1 mM dithiotreitol (pH 7.4). Microsomal fraction and supernatant were obtained by differentiated centrifugation. Four animals were examined per experimental point. The resultant material was examined immediately or stored at -70°C.

Enzyme activities were measured by spectrophotometry: glutathione-S-transferase at 30°C [6] using 1-chloro-2,4-dinitrobenzene as the substrate, NAD(P)H-quinone oxidoreductase at 25°C [4] with 2,6-dichloroindophenol as the electron acceptor, and aldehyde dehydrogenase at 25°C [8] with benzaldehyde as the substrate and NADPH as the electron acceptor. SOD activity was measured at 25°C [3] by inhibition of NBT reduction at 560 nm with superoxide anion radical generated in the xanthine-xanthine oxidase system. The amount of enzyme needed for 50% inhibition of the reaction was taken as a unit of SOD activity. Glutathione peroxidase activity was measured at 25°C by production of oxidized glutathione (NADPH expenditure) at 340 nm with $H_2O_2$ or tertbutyl hydroperoxide (TBHP) as the substrate. The amount of enzyme needed for oxidation of 1 μmol reduced glutathione per min was taken as a unit of glutathione peroxidase activity [1]. Catalase activity was evaluated by the rate of $H_2O_2$ utilization, and protein content by the Biuret method.

## RESULTS

Activities of active oxygen forms detoxication enzymes in tumors were lower than in the liver, irrespective of the site of transplantation. By contrast, the level of phase II enzymes of xenobiotic metabolism was lower in the liver or comparable with that in the tumor. Activity of NADP(H)-quinone oxido- and glutathione reductase was lower in the liver than in the tumor, while the activity of aldehyde dehydrogenase in the liver was comparable with its activity in hepatoma irrespective of the organ of transplantation. Glutathione-S-transferase activity in the liver was intermediate between its levels in hepatoma transplanted subcutaneously and into the liver (Table 1).

Enzyme activities in hepatoma 27 cells transplanted subcutaneously or intrahepatically did not depend on the organ of transplantation. Activities of enzymes involved in detoxication of active oxygen forms were lower in tumors transplanted subcutaneously compared to liver tumors, except catalase whose activity was much higher in hepatoma transplanted subcutaneously. Glutathione reductase activity was higher in hepatoma cells growing in the liver. The function of phase

**TABLE 1.** Activity of Detoxication Enzymes (per mg protein) in the Liver and Hepatoma 27 Transplanted Subcutaneously or into the Liver (*M±m*)

| Enzyme | | Liver | Hepatoma 27 | |
|---|---|---|---|---|
| | | | subcutaneously | in the liver |
| SOD (cytosol), units | | 1478±30 | 505±10 | 298±12 |
| Catalase, μmol $H_2O_2$/min | | 10.29±0.20 | 0.78±0.01 | 3.60±0.01 |
| Glutathione peroxidase, units | $H_2O_2$ | 0.649±0.010 | 0.397±0.040 | 0.244±0.010 |
| | TBHP | 0.622±0.030 | 0.307±0.020 | 0.148±0.020 |
| Glutathione reductase, μmol/min | | 0.169±0.010 | 0.600±0.010 | 0.439±0.010 |
| Glutathione-S-transferase, $10^{-5}$ mmol | | | | |
| | supernatant | 26.73±1.89 | 19.16±1.59 | 27.29±2.96 |
| | microsomes | 12.88±0.14 | 8.01±0.22 | 17.39±0.19 |
| Aldehyde dehydrogenase, μmol/min | | 4449±263 | 4572±175 | 5438±64 |
| Quinone oxidoreductase, $10^{-1}$μmol/min | | 6.60±0.22 | 16.92±1.51 | 25.53±1.96 |

**TABLE 2.** Activity of Detoxication Enzymes (% of Initial Level) in the Liver and Hepatoma 27 Transplanted Subcutaneously or into the Liver after Benz(a)pyrene Injection

| Enzyme | | Liver | Hepatoma 27 | |
|---|---|---|---|---|
| | | | subcutaneously | in the liver |
| SOD | | 87 | 133 | 162 |
| Catalase | | 59 | 609 | 199 |
| Glutathione peroxidase | $H_2O_2$ | 56 | 73 | 142 |
| | TBHP | 55 | 53.7 | 143.7 |
| Glutathione reductase | | 122 | 88 | 136 |
| Glutathione-S-transferase | supernatant | 104 | 203 | 146 |
| | microsomes | 67 | 221 | 87 |
| Aldehyde dehydrogenase | | 139 | 178 | 134 |
| Quinone oxidoreductase | | 134 | 221 | 125 |

II enzymes of xenobiotic metabolism was more effective in hepatoma cells transplanted subcutaneously in comparison with the tumor transplanted into the liver.

Activities of oxygen defense enzymes in cells are known to depend on the level of oxygen: increased oxygen supply activates these enzymes [9]. Rapidly growing tumor tissue is always in a state of hypoxia. Probably that is why activity of oxygen defense system in tumors is lower than in normal liver. Higher activity of these enzymes in the tumor transplanted into the liver in comparison with the tumor transplanted subcutaneously probably means that hypoxia in the tumor growing in the liver is less pronounced than in subcutaneous transplants.

Enzymes involved in detoxication of foreign substances are induced by some xenobiotics, including polycyclic aromatic carbohydrates (PAC). In liver cells NAD(P)H-quinone oxidoreductase is induced, while glutathione-S-transferase activities and aldehyde dehydrogenase are increased negligibly (Table 2). Activities of phase II enzymes of xenobiotic metabolism increased after induction in hepatoma 27 transplanted both subcutaneously and into the liver. Absolute enzyme activities after induction were similar in hepatoma 27 transplanted subcutaneously and into the liver. But constitutive activities in hepatoma cells transplanted into the liver were appreciably lower, and therefore this induction was more pronounced in tumor cells transplanted into the liver than in subcutaneous tumor. Presumably, endogenous inductors forming during subcutaneous growth stimulate the constitutive level of the enzymes. Exogenous inductor increases enzyme activity in subcutaneous tumor to values detected in hepatoma cells transplanted into the liver.

Enzymes involved in oxygen defense in rat liver do not belong to the PAC-inducible enzymes; this agrees with our data. Injection of benz(a)pyrene reduced SOD, glutathione peroxidase, and catalase activities in the liver, while glutathione reductase activity slightly increased (Table 2).

A different picture is observed in the tumor. In hepatoma transplanted into the liver SOD activity slightly increased and catalase activity markedly increased, but did not reach the level in the liver or in tumor transplanted subcutaneously, while the activity of glutathione peroxidase was suppressed. In subcutaneously growing hepatoma, benz(a)pyrene activates all oxygen defense enzymes.

The reaction of oxygen defense enzymes to inductor in the hepatoma transplanted into the liver was more similar to the reaction of normal liver than the reaction of the same enzymes in a subcutaneous hepatoma: glutathione peroxidase activity was suppressed, SOD activity increased though to a less extent than in subcutaneous hepatoma, and only catalase activity increased greatly, but did not reach the level in the liver or in the tumor transplanted subcutaneously. In the tumor transplanted subcutaneously activities of all studied oxygen defense enzymes increased in response to PAC. The inducing effect of benz(a)pyrene can be explained by the fact that in hepatoma cells, in contrast to normal liver, this substance stimulated generation of active oxygen forms, which are the real inductors. However, the exact mechanism of this effect of benz(a)pyrene is unknown. It is not due to the effects of active inductor metabolites, as there is no cytochrome P-450 catalyzing their formation in the liver. Hence, activities of the studied enzymes in tumor cells and their induction in response to injection of benz(a)pyrene are determined by the site of transplantation. The most pronounced difference was observed in the capacity to induction of enzymes of phase II xenobiotic metabolism, which was higher in tumors transplanted into the liver than in subcutaneous tu-

mors. We can assume that transplanted tumor growing in the original organ (hepatoma in the liver) is a model of primary tumor, while a tumor transplanted into a remote organ is a model of metastasis. Our findings confirm this hypothesis: biochemical characteristics of the tumor transplanted into the liver better corresponded to the liver than of a subcutaneously growing tumor.

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