Histochemical Demonstration of Enhanced Glutathione Content in Enzyme-altered Islands Induced by Carcinogens in Rat Liver

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ABSTRACT

The presence of glutathione was demonstrated histochemically in livers of rats treated with diethylnitrosamine or N-nitrosomorpholine. Glutathione content was markedly elevated in adenosine triphosphatase-deficient, \( \gamma \)-glutamyltranspeptidase-positive hyperplastic cell islands. This finding may partly explain the increased resistance of hyperplastic cells to cytotoxic actions of hepatocarcinogens.

INTRODUCTION

Application of hepatocarcinogens to rats leads to the development of hyperplastic cell islands in their livers within 4 to 6 weeks. Loss of ATPase (EC 3.6.1.3) and emergence of \( \gamma \)-GTase have been demonstrated histochemically in these islands (8, 14), which are commonly regarded as putative precursors of liver cancer (4).

An increase in total liver GSH content during the first weeks after the application of various hepatocarcinogens has been demonstrated biochemically (7). GSH is important for detoxification of foreign compounds or their metabolites by means of conjugation (2). It has been suggested that alterations in liver GSH content and \( \gamma \)-GTase activity may be of importance in chemical hepatocarcinogenesis (7). However, a direct correlation between increased GSH content and \( \gamma \)-GTase activity in enzyme-altered cell islands has not been established. Therefore, we have studied histochemically the presence and distribution of GSH in ATPase-deficient, \( \gamma \)-GTase-positive cell islands induced by DEN or N-nitrosomorpholine.

MATERIALS AND METHODS

A group of 8 female Sprague-Dawley rats (inbred strain, Neuherberg), 6 weeks old, received 8 mg DEN per kg body weight per day p.o. for 12 consecutive days. One week later, a commercial mixture of polychlorinated biphenyls (Clophen A 50, 100 mg per kg; Bayer, Leverkusen, Germany) dissolved in olive oil (2 ml per kg body weight) was given once a week for 7 weeks. Due to the promoting effect of polychlorinated biphenyls (10), this dosing schedule resulted in a large number (about 100 to 150 islands per sq cm) of enzyme-altered islands. Two other groups of 4 animals each received either DEN (total dose, 96 mg per kg) or N-nitrosomorpholine (9.1 mg per kg body weight per day p.o. for 15 consecutive days) only without further treatment. For control, 2 animals were completely untreated and 2 received the same amount of olive oil without Clophen A 50 like those of the respective experimental group.

Twelve weeks after the experiment was begun, the animals were starved for 18 hr and killed by cervical dislocation. Liver specimens were frozen at \(-70^\circ\) in isopentane that was precooled by liquid nitrogen. Serial 8-\( \mu \)m cryostat sections were prepared at \(-18^\circ\). Three consecutive sections were used for histochemical demonstration of GSH, ATPase (16), and \( \gamma \)-GTase (12) activity.

GSH was demonstrated with a slight modification of the method described by Ashgar et al. (1). One section was thawed on a glass slide and immediately immersed in a 50 \( \mu \)M solution of mercury orange (Sigma Chemical Co., St. Louis, Mo.) in toluene. After staining for 4 min, the slides were rinsed in toluene (30 sec), dehydrated in ethanol (1 min), immersed in xylene (1 to 2 min), and mounted in Entellan neu (Merck, Darmstadt, Germany). The sections were examined by fluorescence microscopy (Leitz, Wetzlar, Orthoplan fluorescence microscope, equipped for vertical illumination; light source, high-pressure mercury lamp HBO 200, Osram; excitation filter, 2 x KP 490; suppression filter K 510; dichoric mirror K 510). The intensity of fluorescence was measured with a Leitz MPV II microfluorimeter. Mercury orange forms an irreversible complex with the sulfhydryl group of GSH. In sections stained with mercury orange, GSH appears as bright, orange-red granules with a diffuse, yellowish background.

RESULTS AND DISCUSSION

The distribution of GSH in livers from control animals was rather uniform, as described by Ashgar et al. (1). In non-starved animals, the intensity of fluorescence was about 20 to 30% higher than in animals starved for 18 hr prior to killing. This is in accordance with the results of Tateishi et al. (15). In livers from all animals treated with DEN and polychlorinated biphenyls, with DEN only, or with N-nitrosomorpholine, foci were detected which were clearly distinguished from surrounding tissue by their high content of fluorescent granules (Fig. 1). The intensity of fluorescence was about 2-fold higher than in surrounding tissue.

These foci were sharply delineated and coincided in size and shape with the corresponding ATPase-deficient (Fig. 2), \( \gamma \)-GTase-positive (Fig. 3) islands. These findings indicate elevated GSH levels in enzyme-altered hyperplastic islands. The specificity of the staining for GSH was further confirmed by treating animals with diethyl maleate (0.6 ml/kg body weight i.p., 2 hr prior to killing). This treatment has been shown to
lower GSH content in liver to about 10% (3). A marked decrease in GSH staining was observed in livers from these animals. Despite the general decrease of fluorescence in islands as well as in surrounding tissue, especially larger islands were still recognizable.

The conjugation of GSH with a large number of electrophilic compounds as catalyzed by various glutathione-S-transferases is commonly regarded as a very important detoxifying process (8, 11). The protective action of these enzymes, which form about 10% of soluble proteins in rat liver, may be facilitated by high concentrations of GSH. Depletion of GSH may enhance the covalent binding of toxic metabolites to cell macromolecules and the degree of cell damage (13). A high GSH content, as indicated by our results, therefore might protect hyperplastic cells from further impairment by toxic compounds. This suggestion is supported by the increased resistance to cytotoxic agents of enzyme-altered, preneoplastic liver cell populations (6). The resistance to cytotoxic activity is regarded as an important growth advantage of the altered cells over normal hepatocytes in a cytotoxic environment (5, 6).

Future work is necessary to study the increase in GSH content in relation to enzymatic alterations, especially emergence of γ-GTase, and to evaluate the significance of this finding.

REFERENCES


Fig. 1. GSH in enzyme-altered islands of rat liver induced by 8 mg DEN per kg body weight per day for 12 consecutive days and 100 mg polychlorinated biphenyls (Clophen A 50) per kg body weight once a week for 7 weeks. Mercury orange, × 150.

Fig. 2. ATPase-deficiency in the same islands. × 150.

Fig. 3. γ-GTase activity in the same islands. × 150.
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