SELECTION OF γ-GLUTAMYL TRANSPEPTIDASE-POSITIVE HEPATOCYTES AS A FUNCTION OF GSH DEPLETION, OXIDATIVE STRESS AND ALTERATIONS OF INTEGRINS

Johan Högberg and Ulla Stenius
Dept. Toxicol. Institute of Occupational Health
S-171 84 Solna, Sweden

Most carcinogens give rise to cytotoxic effects when administered in doses that are carcinogenic to animals in life time studies (1). There is also ample evidence to suggest that cytotoxicity may promote the carcinogenic process (2,3); in particular, a causal relationship has been discussed in connection with liver carcinogenesis (3). We have become interested in this carcinogenic factor because it tends to influence the risk assessment of many carcinogens (c.f. ref. 4), and a characterization of cytotoxic mechanisms of relevance for the carcinogenic process may lead to a more accurate classification and risk assessment. This mechanistic approach may also stimulate the development of more specific test systems.

We have designed an in vitro model in which cytotoxic chemicals select gamma-glutamyl transpeptidase (GGT) positive hepatocytes, and this model has been used in an effort to define cytotoxic effects which may promote tumor development in the liver (5). Hepatocytes isolated from carcinogen-treated rats (with a high proportion of GGT-positive hepatocytes) have been used. The isolated cells have been exposed to xenobiotics, and have then been allowed to attach to collagen-coated plates (5). It is assumed that GGT-positive cells are mainly derived from enzyme altered foci (EAF) and that they are preneoplastic or have a phenotype similar to preneoplastic cells.

Conditions which have been found to select GGT-positive cells on the plates may be summarized as follows. GSH depletion and oxidative stress, induced by xenobiotics or hydrogen peroxide, leads to a selection of GGT-positive cells (6). Extracellular GSH is essential in most cases, indicating a role for the marker enzyme GGT (5). Of further interest is the indication that a collagen receptor of the integrin family of cell surface receptors seems to be involved in the selection (6). The receptor alteration may suggest that cells in the foci can preserve their competence to grow under conditions in which normal cells cannot respond to growth stimuli. Comparative work has also indicated that there are correlations between results obtained in our model and results obtained in vivo on foci development (7). In this paper the physiological relevance of the model will be further discussed.
MATERIALS AND METHODS

Hepatocytes were isolated from carcinogen-treated rats by collagenase perfusion (5). Isolated cells were then suspended in Krebs-Henseleit buffer and exposed to toxic agents. Samples were withdrawn for measurement of cellular damage (latency of lactic dehydrogenase, LDH) and for attachment studies. In the attachment studies cells were allowed to settle on collagen-coated plates and the cells were then stained for GGT activity. Attached cells were counted and the percentage of GGT-positive cells estimated (5). This latter variable will be referred to as the "GGT-ratio".

RESULTS AND DISCUSSION

Figure 1 shows the kinetics of the selection process induced by hydroquinone in cells from rats that have been pretreated with diethylnitrosamine (DEN) but not with phenobarbital. Previous results have shown that hydroquinone is an effective selector of GGT-positive cells isolated from DEN- and phenobarbital-treated rats (5). The combined regime gives many EAF, but also GGT-positive cells which are not confined to EAF. In order to rule out the possibility that selection occurred only among non-EAF cells the experiment shown in Fig. 1 was performed. The GGT-ratio increased from less than one percent to 6 or 7 percent. Hydroquinone (0.6 mM) induced leakage within three hours, and this was seen as an inability of cells to attach to the plates. Measurements of LDH latency confirmed these observations (data not shown). However, 0.3 mM hydroquinone did not induce an increased leakage during the three hours of incubation. These results indicate that hydroquinone selected for EAF cells, and that this selection may occur in the absence of major toxicological effects. In experiments to be published elsewhere we have also shown that

![Graph showing GGT-ratio vs. time for different concentrations of hydroquinone (HQ).](image)

Fig 1. Hydroquinone (HQ) induced selection of GGT-positive hepatocytes. Hepatocytes isolated from DEN- but not phenobarbital-treated rats were exposed to HQ (at conc. shown in the fig.) and GSH (0.1 mM) in suspension. Cells were withdrawn, washed and then seeded on collagen-coated plates at the times indicated in the Fig. The selection is seen as an increased GGT-ratio (see methods). Samples for leakage measurements were also taken periodically.