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Experimental and Molecular Pathology 81 (2006) 8–14
Experimental and Molecular Pathology
www.elsevier.com/locate/yexmp

Preneoplastic liver cell foci expansion induced by thioacetamide toxicity in drug-primed mice

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Received 6 February 2006
Available online 24 May 2006

Abstract

Mice primed by feeding griseofulvin or diethyl 1,4-dihydro 1,4,6-trimethyl 3,5-pyridine decarboxylate for 5 months followed by drug withdrawal for 1 month (drug-primed mice) were given thioacetamide intraperitoneally, and the livers were subsequently studied at intervals up to 7 days. The hepatocellular proliferative response was measured by immunostaining for proliferative cell nuclear antigen. Necrosis was followed by measuring ALT. Mallory bodies were identified by immunoperoxidase stains for ubiquitin and cytokeratin. Preneoplastic foci were localized using immunofluorescence stain for glutathione *S*-transferase (GST mu) and histochemical stain for gamma glutamyl transpeptidase (GGT). The results showed that the preneoplastic foci selectively proliferated and expanded and formed nodules as indicated by quantitation of nuclei stained positive for proliferating cell nuclear antigen after thioacetamide treatment. Data support the hypothesis that the preneoplastic foci consisted of clones of hepatocytes which preferentially express GST mu, GGT and Mallory bodies. These preneoplastic cells selectively proliferate in response to the promoter effects of necrosis-induced liver cell regeneration ("chemical partial hepatectomy").

Keywords: Preneoplastic foci; Phenotypic marker; Hepatocellular proliferation; Tumor promotion

Introduction

Preneoplastic foci which form Mallory bodies (MBs) are induced by griseofulvin (GF) feeding. Liver nodules and hepatomas subsequently develop in these livers (Tazawa et al., 1983; Cadrin et al., 1990). Refeeding GF or diethyl 1,4-dihydro 1,4,6-trimethyl 3,5-pyridine decarboxylate (DDC) after feeding the drug for 5 months followed by withdrawal of the drug for 1 month (drug-primed mice, DPM) leads to the appearance of preneoplastic foci forming MBs over a 2- to 7-day period (Yuan et al., 1996b). There is rapid induction of liver cell proliferation, induction of gamma glutamyl transferase (GGT) and activation of immediate and late early gene expression, i.e., c-*jun* and c-*myc* and AP-1 activation (Nagao et al., 1998b). AP-1 and NF-κB are activated, and c-*fos*, c-*jun* and c-*myc* expression is increased after 5 months of feeding GF and also in tumors that formed after 16 months of feeding (Nagao et al., 1998a, 1999). In this mouse model of liver carcinogenesis, the same genes are upregulated when the preneoplastic foci stage develop (5 months feeding of the drug) as when tumors developed after 16 months of feeding, i.e., TGFRII, GGT, cytokeratin 8, ubiquitin and cellular transglutaminase. Of these genes, only GGT is significantly more upregulated at 6 months compared with 5 months feeding (Nagao et al., 1999). MBs are induced at 5 months and are seen within the tumors formed at 16 months (Nagao et al., 1999). Thus, the MB forming phenotype is seen in both the preneoplastic foci and the tumors.

The MB forming phenotype has recently been further characterized (Nan et al., 2006) where preneoplastic tumor markers, i.e., FAS, AFP, A2M and GPC-3, are overexpressed in preneoplastic foci. Microarray analysis of the isolated hepatocytes shows global upregulation of growth- and proliferation-related gene expression (Nan et al., 2005). GST is

Abbreviations: GF, griseofulvin; DDC, diethyl 1,4-dihydro 1,4,6-trimethyl 3,5-pyridine dicarboxylate; TA, thioacetamide; GST, glutathione *S*-transferase; GGT, gamma glutamyl transferase; MBs, Mallory bodies; HNs, hyperplastic nodules; DPM, drug-primed mice

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doi:10.1016/j.yexmp.2006.02.006

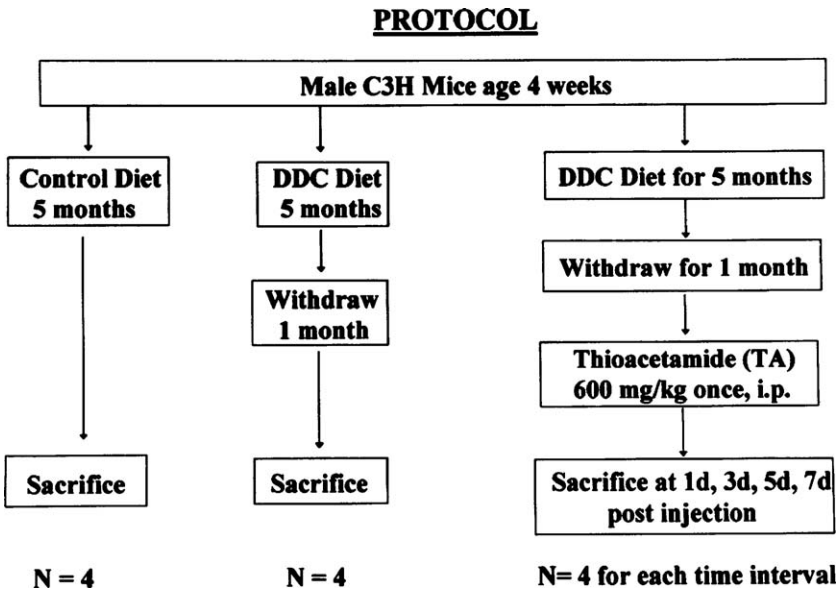

Fig. 1. Experimental design.

overexpressed in preneoplastic foci and hyperplastic nodules at 5 months and tumors at 16 months of griseofulvin feeding (Nagao et al., 1999).

In the present report, the role of tumor promotion in the induction of preneoplastic nodules was studied. Thioacetamide (TA) was used as a promoter and an inducer of glutathione *S*-transferase since it has been shown to induce necrosis followed by liver cell proliferation (Mungipudy et al., 1995). The increase in DNA synthesis following TA-induced necrosis follows a time course which is similar to that seen after partial hepatectomy (Mungipudy et al., 1995). This phenomenon is referred to as "chemical hepatectomy." Thus, TA toxicity can be used to stimulate the growth of preneoplastic nodules over a course of 5 to 7 days and is ideal to promote this response in the drug-primed mouse model (Yuan et al., 1996a,b). Thiacetamide toxicity has been shown to also induce Mallory body formation in these drug-primed mice (French et al., 2001).

The preneoplastic foci studied here resemble the resistant phenotype which selectively proliferates to form a hyperplastic nodule in an altered adaptive response to toxic xenobiotics (Farber, 1990). These foci are composed of clones of hepatocytes which have been genetically altered by previous exposure to carcinogens (Farber, 1990). The clones can be identified histochemically because they express gene products which are not normally overexpressed by hepatocytes such as GGT and glutathione *S*-transferase (GST) (Farber, 1990; Sakaida and Okita, 2005; Nagao et al., 1999). Thus, the resistant phenotype can be identified as the source of hyperplastic nodules by histochemistry and antibodies to proteins expressed in the nuclei during DNA synthesis such as proliferating cell nuclear antigen (PCNA). Therefore, to test this hypothesis, the drug-primed mice were subjected to a sublethal dose of thioacetamide (600 mg/kg, ip) and the

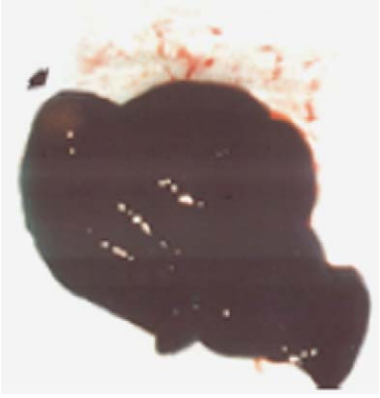

Fig. 2. Gross photograph of a liver nodule (arrow) from a DPM mouse 7 days after TA treatment. The liver is dark brown color due to protoporphyrin pigment deposits.

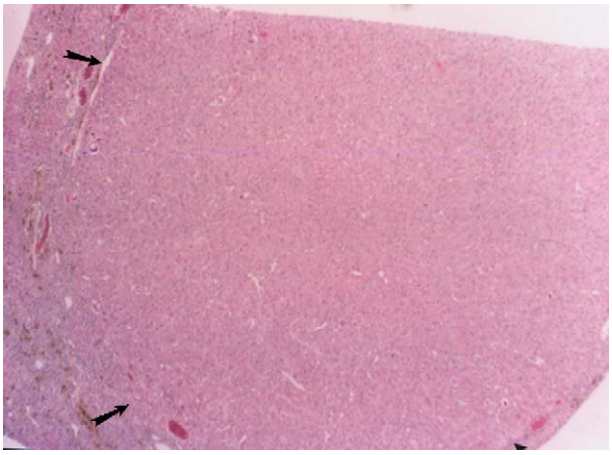

Fig. 3. Microscopic liver nodule that was found 5 days after TA treatment. The edge of the nodule is marked by arrows. Hematoxylin and eosin. ×156.