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Cancer Letters 217 (2005) 25–32
CANCER Letters
www.elsevier.com/locate/canlet

Oxidative stress in carcinogenesis. Correlation between lipid peroxidation and induction of preneoplastic lesions in rat hepatocarcinogenesis

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Received 1 June 2004; received in revised form 1 July 2004; accepted 6 July 2004

Abstract

Oxidative stress during carcinogen metabolism seems to participate in liver tumor production in the rat. *N*-diethylnitrosamine is an important carcinogen used in liver cancer animal models. This indirect alkylating agent produces DNA–ethyl adducts and oxidative stress. In contrast, *N*-ethyl-*N*-nitrosourea, a direct mutagen, which generates DNA–ethyl adducts, does not produce liver tumors in rat unless it is given under oxidative stress conditions such as partial hepatectomy or phenobarbital treatment. To gain insight into the relation between oxidative stress and hepatocarcinogenicity, the induction of preneoplastic liver lesions was compared among three different initiation protocols related to the initiation–promotion-resistant hepatocyte model. In addition, liver lipid peroxidation levels, determined as thiobarituric acid reactive sustances were studied early during the initiation stage. Rats initiated with *N*-ethyl-*N*-nitrosourea, 25 days after treatment developed fewer and smaller γ-glutamyl transpeptidase positive preneoplastic lesions than rats initiated with *N*-diethylnitrosamine. A pre-treatment with the antioxidant quercetin 1 h before *N*-diethylnitrosamine initiation, significantly prevented development of γ-glutamyl transpeptidase-positive lesions. Increased lipid peroxidation levels were induced with *N*-diethylnitrosamine from 3 to 24 h after initiation, while *N*-ethyl-*N*-nitrosourea did not induce increments, and importantly, pre-treatment with quercetin decreased lipid peroxidation induced by *N*-diethylnitrosamine. These results show correlation between lipid peroxidation and hepatocarcinogenicity and support the important role of oxidative stress on liver carcinogenesis.

Keywords: Oxidative stress; Initiation; Lipid peroxidation; Preneoplastic lesions; Hepatocarcinogenesis

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doi:10.1016/j.canlet.2004.07.019

1. Introduction

There are clear examples of the participation of reactive oxygen species (ROS) in hepatocarcinogenesis in the rat. Nakae et al. [1] showed that initiation with low doses of *N*-diethylnitrosamine (DEN) induced liver DNA-8-hydroxydeoxy-guanosine adducts and suggested that oxidative stress participates in hepatocarcinogenesis. Therefore, one can assume that initiation with high doses of DEN produces oxidative stress, as in the case of hepatocarcinogenic models such as that by Solt and Farber, initiated with DEN and promoted with 2-acetylaminoflourene (2-AAF) and partial hepatectomy (PH) as proliferative stimulus [2], or modifications of this, as in the Semple-Robert's [3] model. During a choline-deficient diet, the representative marker of oxidative DNA damage 8-hydroxy-deoxy-guanosin is induced [4] and the same is true after administration of cipofibrate, one of the more efficient peroxisome proliferators that induces liver cancer in the rat [5]. Trimethylarsine oxide, an organic metabolite of inorganic arsenics, produced liver tumors in male Fischer 344 rats and authors implicate a possible mechanistic role of oxidative DNA damage and enhanced cell proliferation [6]. Also, in male Fischer 344 rats, it was demonstrated that α,α-bis(*p*-chlorophenyl)-β,β,β trichloroethane (DDT) induces eosinophilic foci and hepatocellular carcinoma (HCC) as a result of oxidative DNA damage [7].

It is not clear yet if participation of oxidative stress depends on the DNA oxygen adducts or if there is a concomitant alteration of signalization by the abrupt induction of ROS during initiation, or if both, DNA damage and a new intracellular reduced steady state are necessary for carcinogenesis to take place. Evidence with U937 cells treated with two well-known nitrosamines, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and DEN showed that ROS are produced; these activate nuclear factor κB (NF-κB); subsequently, cyclooxygenase-1 (COX-1) activity is induced, and this pathway increases prostaglandin E_2 (PGE_2) synthesis [8]. These results, and evidence that acetylsalicylic acid, a non-steroidal anti-inflammatory drug, and NNK lung carcinogenesis inhibitors block activation of NF-κB, induction of COX-1 and PGE_2 synthesis, together lend support to the proposition that ROS participate in carcinogenesis. When NNK or DEN were substituted by their respective *O*-acetate derivatives, which do not need to be metabolized, they did not activate NF-κB or induce PGE_2 synthesis, even though it is known that these nitrosamines as well as their acetates produce DNA adducts [8,9].

There is evidence that oxidative stress is an obligatory component of carcinogenesis. It was recently communicated that COX-1 or COX-2-deficient mice had altered epidermal differentiation and, when treated with 7,12-dimethylbenz(a)anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-12,13-acetate (TPA), they presented reduced skin tumorigenesis, although DMBA stable DNA adducts were increased twice [10]. This study clearly shows that there is no correlation between adduct levels and tumorigenesis. Even though the authors do not demonstrate that oxidative stress is produced, a direct relation between COX-1 and COX-2 deficiency is hypothetically associated to a lesser degree of oxidative stress [10].

We propose that, in the rat hepatocarcinogenesis model, both direct alkylating DNA damage and alterations produced by ROS induction during carcinogen metabolism are necessary processes for liver cancer induction. The dilemma is how to differentiate the participation of DNA alteration by ethyl adducts [9] from the participation of cell modifications induced by ROS or, even more, from the obliged hypothetical participation of both to induce initiation. To gain insight into this problem, experiments were carried out to compare (a) induction of gamma-glutamyl transpeptidase-positive (GGT^+) preneoplastic lesions on the 25th day, as an early end point of hepatocarcinogenesis in a DEN-2AAF-PH model or (b) in the same model, substituting DEN by *N*-ethyl-*N*-nitrosourea (ENU), a direct carcinogen, that is only carcinogenic in the rat liver under very special circumstances [11–13] or (c) by administration of quercetin, an antioxidant, administered previous to the DEN, 2AAF treatment. And as a key comparative feature, in these three different groups, LPX was measured during the initiation period.

2. Methods

2.1. Reagents and animals

All reagents were purchased from SIGMA (St Louis, MO, USA). Male Fischer-344 rats