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Chemopreventive effect of bicyclol on malignant transformation of WB-F344 rat liver epithelial cells and its effect on related signal transduction in vitro

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Abstract

The preventive effect of bicyclol, a novel anti-hepatitis drug, on hepatocarcinogenesis and its mechanism of action was studied in vitro. The results clearly indicated that bicyclol at non-toxic doses prevented the malignant transformation of WB-F344 cells (WB cells) induced by 3-methylcholanthrene (3MC) and 12-*O*-tetradecanoyl phorbol 13-acetate (TPA). Furthermore, bicyclol inhibited proliferation of quiescent WB cells stimulated by TPA and blocked TPA-induced down-regulation of the gap junctional intercellular communication (GJIC). Immunoblot analysis demonstrated that bicyclol exhibited a remarkable reversing effect on TPA-induced cPKC-α and phosphor-ERK1/2 expressions. In addition, bicyclol attenuated TPA-induced IκB-α degradation. In conclusion, our results support that bicyclol has chemopreventive action against hepatocarcinogenesis through inhibition of related signal transduction.

Keywords: Bicyclol; Liver cancer; Chemoprevention; Rat liver epithelial cell; Gap junctional intercellular communication

1. Introduction

Human hepatocellular carcinoma (HCC) is one of the most frequent malignant cancers. Multiple etiological factors are involved in the development of HCC, and the most frequent causes of HCC are chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections [1]. About 80% of human HCC are attributable to HBV infection [2]. Chronic HBV carriers are 100–400 times more likely to develop HCC than non-carriers [3]. HCV is the second most common cause of HCC after HBV [4]. Now, HCC represents more than 4% of all cancer cases in the world and causes at least 315,000 deaths every year [5]. Although early HCC can be cured by surgical resection, many HCC are asymptomatic, so most of HCC patients are not diagnosed in time.

An effective approach to cancer control is chemoprevention. It is known that the therapy of both chronic HBV and HCV needs generally a course of long term. If an anti-hepatitis drug has inhibiting or

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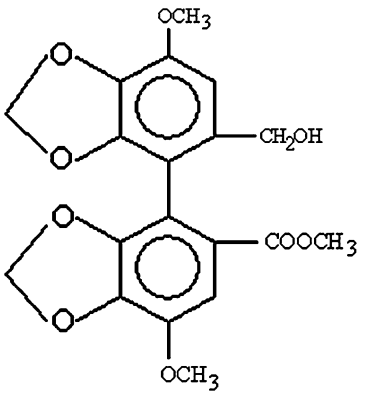

Fig. 1. The chemical structure of bicyclol.

suppressing effect in the development of hepatocarcinogenesis besides its improvement of abnormal liver function and inhibition of hepatitis virus replication, this kind of drug would be of great clinical value.

Bicyclol(4,4′-dimethoxy-2,3,2′,3′-dimethylenedioxy-6-hydroxymethyl-6′-carbonyl-biphenyl) is a novel anti-hepatitis drug developed by Chinese scientists (Fig. 1) [6]. Clinical trial found that bicyclol is effective in improvement of the abnormal liver function, and also effective in inhibiting the replication of hepatitis B virus in chronic hepatitis B patients [7]. Pharmacologically, bicyclol has protective action against liver injury induced by hepatotoxins in mice and rats, anti-fibrotic effect in CCl_4-induced liver fibrosis in rats and mice, and anti-hepatitis virus action in duck viral hepatitis and 2.2.15 cell line [6,8,9]. Furthermore, bicyclol induced differentiation of human hepatocarcinoma HepG2 and Bel7402 cells and reduced AFB1 hepatotoxicity by increasing the detoxifying metabolism of AFB1 in rat liver [10]. The results implicate the possibility that bicyclol have chemopreventive effect on liver carcinogenesis.

The purpose of the present paper was to study the chemopreventive effect of bicyclol on hepatocarcinogenesis and its effect on related signal transduction in vitro. For this purpose, a two-stage chemical oncogenesis model was established in WB-F344 rat liver epithelial cells (WB-F344 cells) with 3-methylcholanthrene (3MC) and 12-*O*-tetradecanoyl phorbol 13-acetate (TPA). The effects of bicyclol on cell transformation, proliferation, gap junctional intercellular communication (GJIC) and the signal transduction pathways related to carcinogenesis were also assessed.

2. Materials and methods

2.1. Chemicals

Bicyclol with 99% purity was provided by the Beijing Union Pharmaceutical Plant. As bicyclol is not water-soluble, it was dissolved in dimethyl sulfoxide (DMSO) for in vitro use. Lucifer yellow CH, 3-methylcholanthrene (3MC), 12-*O*-tetradecanoyl phorbol 13-acetate (TPA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetraz-olium bromide (MTT), dithiothreitol (DTT), propidium iodide (PI), phenylmethanesulphonyl fluoride (PMSF), aprotinin, leupeptin, *N*′*N*-methylene-bis-acrylamide and acrylamide were purchased from Sigma Chemical Co. Other chemicals were of analytical grade and purchased from Beijing Chemical Company.

2.2. Cell culture

WB-F344 cells were grown in DMEM (GIBCO) media containing 10% newborn calf serum, 100 unit/ml penicillin and 100 μg/ml streptomycin at a 37 °C humidified incubator containing 5% CO_2 and 95% air, and passaged by 0.25% trypsin plus 0.02% EDTA treatment. The culture medium was changed every other day.

2.3. Cytotoxicity assay

Cytotoxicity was determined by MTT assay and colony formation assay.

MTT assay. According to the method of Mosmann [11], WB-F344 cells (3×10^3 cells/well) were plated on 96-well plates in the complete medium, 24 h later, various concentrations of bicyclol were added (1×10^{-6}–5×10^{-4} M). The cells were incubated at 37 °C in a CO_2 incubator for 72 h. The culture supernatant was sucked out, 100 μl of 0.5 mg/ml MTT stock solution in free-serum medium was added to each well. After 4 h of incubation, 150 μl of DMSO was added. The plates were mixed gently until the blue sedimentation was completely dissolved. The optical density of each well was determined by a microplate