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Possible role of selective, irreversible, proteasome inhibitor (carfilzomib) in the treatment of rat hepatocellular carcinoma

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ABSTRACT

We investigated the possible therapeutic effect of irreversible proteasome inhibitor, carfilzomib against hepatocellular carcinoma induced chemically by chronic administration of diethylnitrosoamines (DENA).

Hepatocellular carcinoma induced by DENA in male Wistar rats was manifested biochemically by significant elevation of serum α-feto protein (AFP) and carcinoembryonic antigen (CEA). In addition, hepatic cancer was further confirmed by a significant increase in hepatic tissue growth factors; vascular endothelial growth factor (VEGF), transforming growth factor-β1 (TGF-β1) and basic fibroblast growth factor (FGF). Moreover a marked increase in matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-1 (TIMP-1) content were also observed, along with a profound decrease in hepatic endostatin and metallothionein level.

Treatment of rats with the selected doses of carfilzomib produced a significant protection against hepatic cancer. The present results claimed that chosen doses of carfilzomib succeeded in suppressing serum tumor markers level AFP and CEA. Furthermore, the drug reduced the elevated level of hepatic growth factors, MMP-2 and TIMP-1 induced by the carcinogen. The antitumor effect of carfilzomib was also accompanied by augmentation of hepatic content of endostatin and metallothionein. Histopathological examination of liver tissues also correlated with the biochemical observations. It could be concluded that treatment with carfilzomib confers a possible antitumor effect against hepatocellular carcinoma induced by DENA model in rats.

1. Introduction

Human cancers treatment is limited by the unwanted adverse effect of chemostatic or chemotoxic anti-neoplastic agents. Hepatocellular carcinoma (HCC) is the most common type of liver cancer in the world with an estimated annual incidence of greater than 1 million new cases per year [1]. Up to now, there is no standardized, very effective approach for the treatment of inoperable HCC. Alternatively, several agents with more efficacies have been employed in treating HCC, however, its long term therapeutic outcome remains very poor [1]. The most commonly systemic chemotherapeutic agents are doxorubicin and 5-fluorouracil [2]. However these drugs are quite toxic and the results remain disappointing [2].

Changes in proteasome functions have been directly involved in the etiology of many cancers [3]. In general, specific malignancies can result from elevation of oncoproteins or breakdown of tumor suppressor genes products. Inhibition of proteasome functions has a potential antitumor activity [4].

Based on the great success achieved by application of bortezomib as a novel treatment for multiple myeloma (MM), a number of next-generation of proteasome inhibitors have been developed with the aims of improving efficacy, overcoming drug resistance, minimizing dose-limiting toxicity such as peripheral neuropathy (PN) and improving convenience of administration [5]. The recent accelerated approval of carfilzomib (tetrapepetide epoxyketone) is an example the success of this approach. The first study investigated the effect of proteasome inhibitors (PIs) in HCC was in 2004 demonstrating that MG-132 induced apoptosis in human HCC cells through caspase cascade leading to β-catenin cleavage and down-regulation of β-catenin-mediated transactivation [6].

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To investigate the possible antitumor effect of carfilzomib on liver cancer, we used the DENA for induction of rat hepatocellular carcinoma model. DENA is a well-known potent hepatocarcinogenic agent present in tobacco smoke, water, cured and fried meals, cheddar cheese, agricultural chemicals, cosmetics and pharmaceutical products [7–9]. DENA is known to induce damage in many enzymes involved in DNA repair and is normally used to induce liver cancer in experimental animal models [10]. After its use, many studies have investigated a series of microscopic lesions called "foci" and "nodules" which have been designated "preneoplastic" or "premalignant" [11]. During the process of neoplastic transformation, various histochemical and biochemical marker enzymes and protein antigens are expressed depending upon the stages and magnitude of neoplasia. These markers are frequently considered as surrogate end-point biomarkers in rat liver carcinogenesis model [12].

To date and up to our knowledge, there are no published studies investigating the possible effect of irreversible proteasome inhibitor; carfilzomib against hepatic cancer induced by DENA. Therefore, the present work was undertaken to investigate the possible antitumor effect of different doses of carfilzomib. Moreover, to identify the underlying mechanisms by studying the effect of selected doses of the drug on the different hepatic growth factors, MMP-2, TIMP-1, endostatin, and metallothionein as an index of antioxidant status.

2. Materials and methods

2.1. Animals

Adult male albino rats of the Wistar strain (170–200 g) were obtained from Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia. The animals were housed in cages under standard controlled environmental hygienic conditions (25 °C and a 12 h light/dark cycle). Animals have free access to pulverized standard rat pellet diet and fed chow spruce and water ad libitum. All animal procedures followed the international guidelines of proper experimental animal handling.

2.2. Chemicals

Diethylnitrosoamine will be obtained from Santa Cruz Biotechnology, Inc. 2145 Delaware Avenue Santa Cruz, CA. 95060 USA. Carfilzomib was obtained from Active Biochem. Redan, GA 30074 USA, it was freshly dissolved in dimethylsulphoxide (DMSO) prior to injection. Matrix metalloproteinase-2 (MMP-2), tissue inhibitor of metalloproteinase-1 (TIMP-1), vascular endothelial growth factor (VEGF), transforming growth factor-β1 (TGF-β1) and basic fibroblast growth factor (FGF) were purchased from R&D Systems, Inc. USA. Endostatin, metallothionine, carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP) were obtained from Uscnlife Science & Technology Co. Ltd (Guanguguoji, East Lake Hi-Tech Zone, and Wuhan, China). All other chemicals used were obtained from Sigma and were of high analytical grade.

2.3. Experimental protocol

The animals were divided randomly into 6 groups, 10 animals in each group. The first group (control) received vehicles used for carfilzomib, DMSO (0.4 ml/kg i.p.) twice a week from week 16 to week 18. The second and third groups received carfilzomib (2 and 4 mg/kg i.p.) twice a week from week 16 to week 18, respectively [13]. The fourth group was given DENA at a dose of 0.01% in drinking water for 15 weeks, the calculated dose was based on the average daily intake of water for each rat and received a DMSO (0.4 ml/kg i.p.) twice a week from week 16 to week 18 [14,15]. The last two groups were given DENA at a dose of 0.01% in drinking water for 15 weeks [13] and treated with carfilzomib (2 and 4 mg/kg i.p.) twice a week from week 16 to week 18, respectively [13]. The selected concentrations of the drugs and the schedule of dose administration were chosen as guided by our own preliminary experiments. At the end of treatment protocol, the blood samples were taken by cardiac puncture, under light ether anesthesia, into non-heparinized tubes. Serum was separated by centrifugation for 5 min at 1000*g* and stored at −20 °C until analysis. Animals were sacrificed by cervical dislocation and the liver was quickly isolated, washed with saline, blotted dry on filter paper and weighed. A 10% (w/v) homogenate of the liver tissues was prepared in ice cold saline using a Branson sonifier (250, VWR Scientific, Danbury, Conn., USA).

2.4. Assessment of biochemical parameters

2.4.1. Enzyme linked immunosorbent assay (ELISA) of AFP and CEA

Quantitative estimation of hepatic tumor markers AFP and CEA were based on ELISA using assay kits from Uscnlife Science & Technology CO. LTD (Wuhan, China) according to manufacturer's instructions.

2.4.2. Enzyme linked immunosorbent assay (ELISA) of different growth factors, matrix metalloproteinase-2, tissue inhibitor of metalloproteinase-1, endostatin, and metallothionein levels in liver homogenates

VEGF, TGF-β1, FGF, MMP-2 and TIMP-1 were assayed in the liver homogenates by ELISA using assay kits from R&D systems (Minneapolis, MN) according to manufacturer's instructions. In addition, endostatin, metallothionein were also analyzed by ELISA using assay kits from Uscnlife Science & Technology Co. Ltd (Wuhan, China) according to manufacturer's instructions.

2.5. Histopathological evaluation

Histopthological evaluation was performed on the liver and a portion of the specimen was fixed in 10% formalin and embedded in paraffin wax. Sections were cut at 4 μm thicknesses, stained with hematoxylin and eosin and viewed under light microscope. To avoid any type of bias, the slides were coded and examined by two histopathology's who were blinded to the treatment groups. It was investigated for the presence of hepatic cirrhosis, hepatocellular dysplasia (dysplastic cirrhotic nodules) and frank hepatocellular carcinoma formations. The size of malignant hepatic foci in six rats from DENA-intoxicated and carfilzomib treated hepatic cancer induced by DENA intoxication groups were measured using a microscopic eye piece graticule. Measurements were done on three sections taken from each animal liver. A high power Nikon Eclipse 80i objective (40×) was used and measurements were done using the eye piece graticule on 0.59 mm field diameter space.

2.6. Statistical analysis

Data are expressed as means ± SEM (n = 10). Statistical comparison between different groups were done using one way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison tests, to judge the difference between various groups. Significance was accepted at $P < 0.05$.