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The effectiveness of cyclooxygenase-2 inhibitors and evaluation of angiogenesis in the model of experimental colorectal cancer

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ABSTRACT

Colorectal cancer (CRC) is an important cause of cancer-related deaths worldwide. Early diagnosis and treatment of CRCs are of importance for improving the survival. In the present study, we studied the effects of nonsteroidal anti-inflammatory drugs (NSAIDs)-induced chemopreventive effects on tumor development incidence and angiogenesis in experimental CRC rats. 1,2-Dimethylhydrazine dihydrochloride (DMH) was used as cancer-inducing agent and two NSAIDs (celecoxib and diclofenac) were given orally as chemopreventive agents. Histopathological and immuno histochemical evaluations were performed in colorectal tissue samples, whereas angiogenesis parameters were studied in blood samples. Histopathological examination showed that adenocarcinoma (62.5%), dysplastic changes (31.25%) and inflammattory changes (6.25%) were detected in DMH group, whereas no pathological change was observed in control rats. In treatment groups, there was marked decrease in adenocarcinoma rate (30% and 10%, respectively). A significant increase was detected in MMP-2, MMP-9 levels and MMP-2/TIMP-2 ratio in DMH group as compared with controls and treatment groups. In immunohistochemical evaluations, there was an increase in intensity and extent of staining of MMP-2 and MMP-9 in DMH group as compared to controls and treatment groups. The decrease in celecoxib group was more prominent.

Overall, it was concluded that NSAIDs, particularly cyclooxygenase-2 (COX-2) inhibitors, might have a protective effect on CRC development and slow down progression of tumor in a DMH-induced experimental cancer model. One of the possible mechanisms in the chemoprevention of colon cancer seems to be inhibition of angiogenesis by diclofenac and celecoxib.

1. Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer around the world, and the third important reason of cancer-related deaths for men and women in the USA [1,2]. This prevalence has turned colorectal cancer into an important health problem around the world. CRC is a complex process of which pathogenesis includes many molecular pathways. This process comprises the many effects including interactions related to environmental factors, genetic characteristics on personal cancer tendency and the accumulation of the somatic changes in the colorectal epithelium [3]. Matrix metalloproteinases (MMPs) and tissue specific matrix metalloproteinase inhibitors (TIMPs) have crucial roles in many stages of the tumoregenesis such as inflammation, transition from adenoma to carcinoma, angiogenesis, invasion and metastasis [4,5]. MMPs are zinc-dependent endopeptidases with the ability to degrade the extracellular matrix (ECM), certain basement membrane proteins and extracellular adhesion molecules [6]. In addition to be active in biological activities in the morphological development of the tissues such as cell proliferation, differentiation and migration, the ECM is regarded as a primary barrier in the prevention of the growth and the spread of the tumoral tissue. Tumor cells use the metalloproteinases in order to overcome this barrier [7].

Gelatinase group members of matrix metalloproteinases, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) have the ability to destruction the gelatines and elastin with type-4 and 5 collagens [8]. Non-specific inhibitors and specific TIMPs [9] suppress proteolytic activities of the MMPs. TIMPs are synthesized like MMPs in vascular smooth muscle cells, endothelial cells, blood cells, *connective* tissue cells and macrophages. Proteolytic remodeling of the ECM is an important component of disease progression in many chronic disease states and the imbalance between MMPs and TIMPs is associated with the onset of the tumor microenvironment in cancer, particularly during cancer progression and metastasis [10,11]. In this purpose, MMPs can be used for early diagnosis of cancer, detecting and monitoring the progression of

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metastasis [12]. MMP-2 and MMP-9 have been shown to be increased in CRC by several studies and their expression may be used as potential biomarkers [13–15].

The hallmarks of cancer comprise various biological capabilities acquired during the multistep development of human tumors. The hallmarks of cancer include sustaining proliferative signaling, evading growth suppressors, resisting cell death (apoptosis inhibition), enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, genome instability and mutation, and inflammation which associated with tumor [16,17]. In fact, these acquired skills represent abnormalities in several stages the control mechanisms associated with cell survival, proliferation, migration, invasion, up to interact with neighboring cells and stroma, and can be summarized in this way [18]. Many therapeutic targets have been developed using this properties. One of these targeted therapies is use of selective nonsteroid antiinflammatory drugs (NSAIDs) against the tumor-associated inflammation [17]. In recent years, significant results about the positive effects of selective/specific cyclooxygenase-2 (COX-2) inhibitors such as celecoxib and diclofenac with nonsteroidal anti-inflammatory properties on CRCs were obtained in many studies [19–24].

The aim of the present study is to investigate on angiogenesis process, which is effective in MMP-2, MMP-9 and TIMP-2 levels (a natural inhibitor of metalloproteinases) in 1,2-Dimethylhydrazine (DMH)-induced colon cancer model and evaluated the implementation of COX-2 inhibitors for molecular targets on tumor considering the above-mentioned mechanisms.

2. Materials and methods

2.1. Animal procurement

Male Sprague-Dawley rats weighing 200–250 g (8 weeks old) were obtained from the Firat University Experimental Research Center, Elazığ. The animals were acclimatized for at least 1 week, and were given normal diet (rodent chow) and water ad libitum. They were housed three-four per cage in polypropylene cages with a wire mesh top and a hygienic bed of husk (regularly changed) in a well-ventilated animal room until the end of the experimental period. The animals were also maintained at ambient temperature of 22–24 °C, in automatically climatized room and under a 12 h light/dark period. They were maintained according to the principles and guidelines of the Ethics Committee of Animal Care of Firat University (number 11/100, 08.11.2012). Body weights of the animals, hair loss, defecation defects and anal lesions were evaluated and recorded.

2.2. Chemicals and preparation

DMH was purchased from Sigma Aldrich (St. Louis, MO, USA). DMH was freshly prepared in 1 mM EDTA-saline and pH was adjusted to 7.0 using dilute NaOH solution [25]. Doses of NSAIDs used were determined based on the reported therapeutic anti-inflammatory dose (ED50 for rats) values in the therapeutic dose range. The determined values for diclofenac (Dikloron tablet, Deva Holding A.Ş, Turkey) was 8 mg/kg body weight [25] and celecoxib (Santa Cruz Biotechnology, USA) was 6 mg/kg body weight [19].

Diclofenac and celecoxib were dissolved in dimethyl sulfoxide (DMSO) 10%, then were diluted with distilled water. Freshly prepared treatment agents were administered at 1 mL/day throughout the test period by orogastric gavage. During the drug applications, a short-term sedation was induced by inhalation of ether to provide ease of application and not to irritate rats in order.

2.3. Protocol relating to the establishment of the experimental cancer models with DMH

A dosage of 25 mg/ kg body weight DMH was injected subcutaneously (sc) once a week for 12 weeks. Injections were performed as rotation from rat lumbar level of the back. Thirteen weeks after the last DMH injection was defined as latent period and expected to occur in neoplastic transformation. Thus, whole experimental protocol was completed within 25 weeks.

2.4. Experimental design

Forty-eight male Sprague-Dawley rat pups were randomly divided into a control group and 3 experimental groups.

Rats in the control group (n = 8) received 1 mM EDTA saline subcutaneously (sc) via weekly injection for 12 weeks and DMSO per os (po) at 1 mL/day throughout experiment (25 weeks).

In experimental groups, the rats were randomized into 3 groups.

Rats in the DMH-induced-colorectal cancer group (n = 16) were administered 25 mg/ kg body weight DMH (sc) once weekly for 12 weeks, 1 ml DMSO (po, daily) for 25 weeks, and termed the DMH group.

DMH + diclofenac Group (n = 12) received 25 mg/ kg body weight DMH (sc) once a week for 12 weeks, and 8 mg/kg body weight diclofenac was given daily throughout the test period (25 weeks).

DMH + celecoxib Group (n = 12); celecoxib was given daily within its therapeutic anti-inflammatory dose (ED50 for rats, 6 mg/kg body weight) for 25 weeks along with the weekly administration of 25 mg/kg body weight of DMH for 12 weeks.

At the end of this study, the animals were kept on overnight fasting with drinking water ad libitum and sacrificed the next day using sterile instruments under an over anesthesia with ether. The weights of animal body in all the groups were recorded once in a week until the end of the experiment. Growth rate was calculated by using the following formula:

Growth rate = Final body weight − Initial body weight/Total number of experimental days

2.5. Collection and preparation of sample

Blood samples were collected in EDTA tubes and then were centrifuged at 3000 rpm for 10 min at 4 °C, and the plasma was obtained which was kept in −80 °C until biochemical assays were performed. They were used for the determination of MMP-2, MMP-9 and TIMP-2.

2.6. Elisa assays

Plasma MMP-2 (Boster, Boster Biological Technology Ltd. Catalog no: EK0639, USA), MMP-9 (EastBiopharm, Hangzhou EastBiopharm CO. Ltd. Catalog no: CK-E30434, China) and TIMP-2 (EastBiopharm, Hangzhou EastBiopharm CO. Ltd. Catalog no: CK-E30522, China) measurements were performed according to the manufacturer guidelines by using commercially available solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) kit.

2.7. Macroscopical analysis

Complete necropsy was performed and then colon and rectum were removed without damage to the colon tissue. Then, colon was flushed out with ice phosphate buffered saline to remove the faecal matter and other debris. After thorough checking for free flow of saline from one end to the other, the cleaned colons were cut longitudinally and placed on a tissue paper and photographed. Number tumors were documented.The number, location and size of each tumor were scored by an independent observer. Endpoints were colon tumor incidence, tumor multiplicity (number of tumors/rat) and tumor size (tumor size/rat).

2.8. Histopathological analysis

The colons were divided into proximal, medial and distal segments