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Original Articles

Aldose reductase inhibition suppresses azoxymethane-induced colonic premalignant lesions in C57BL/KsJ-db/db mice

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ABSTRACT

Type-2 diabetes and obesity-related metabolic abnormalities are major risk factors for the development of colon cancer. In the present study, we examined the effects of polyol pathway enzyme aldose reductase (AR) inhibitor, fidarestat, on the development of azoxymethane (AOM)-induced colonic premalignant lesions in C57BL/KsJ-db/db obese mice. Our results indicate that fidarestat given in the drinking water caused a significant reduction in the total number of colonic premalignant lesions in the AOM treated obese mice. Further, the expression levels of PKC-β2, AKT, COX-2 and iNOS in the colonic mucosa of AOM-treated mice were significantly decreased by fidarestat. The serum levels of IL-1α, IP-10, MIG, TNF-α and VEGF are significantly suppressed in AOM + fidarestat treated obese mice. Fidarestat also decreased the expression of COX-2, iNOS, XIAP, survivin, β-catenin and NF-κB in high glucose-treated HT29 colon cancer cells. In conclusion, our results indicate that fidarestat inhibits the development of colonic premalignant lesions in an obesity-related colon cancer and is chemopreventive to colorectal carcinogenesis in obese individuals.

Introduction

Colorectal cancer (CRC) is a key health problem worldwide. Several evidences indicate that the risk of CRC is increased in patients with metabolic syndrome, also called insulin resistance syndrome, which is usually associated with obesity and related metabolic defects [1,2]. Obesity is the leading cause of insulin resistance and hyperinsulinemia, which is also a likely risk factor for CRC [3]. Several hypotheses have emerged to explain the influence of obesity on the development of CRC, including insulin resistance, alterations in the insulin-like growth factor (IGF)/IGF-1 receptor axis and adipocytokine imbalance, such as increased leptin levels and decreased adiponectin levels [4–7]. ROS-mediated inflammation induced by excessive production of storage lipids, high circulating glucose levels and pro-inflammatory cytokines produced by adipose tissue plays a critical role in obesity- and diabetes-related colorectal carcinogenesis [4,8–11]. These reports suggest that targeting ROS-induced inflammation may provide an effective strategy for preventing the development of CRC.

Our recent studies in human colon cancer cells, human lens epithelial cells, vascular endothelial cells and vascular smooth muscle cells suggest that the polyol pathway enzyme, aldose reductase (AR), a member of aldo–keto reductase super family, is a regulator of ROS signals induced by growth factors, cytokines, chemokines, lipopolysaccharide and high glucose (HG) [12–19]. Overexpression of AR has been shown in the tissues of diabetic and obese subjects [20,21]. Further, AR has been shown to be overexpressed in a number of human tumors [22]. In human colon cancer Caco-2 cells, we have shown that inhibition of AR prevents the cytokines- and growth factor-induced COX-2 expression, activation of NF-κB and PGE2 production [12]. Further, our studies have also shown that inhibition of AR prevents tumor growth in nude mice xenografts, and CRC metastasis in mice [16–18]. An early event in the development of colon cancer is the formation of preneoplastic lesions called aberrant crypt foci (ACF). We have also shown that AR inhibition prevents ACF formation in azoxymethane-treated BALB/c mice [19]. Since, obesity and hyperglycemia are the main risk factors for the development of colon cancer, we investigated the role of AR in azoxymethane (AOM)-induced ACF in C57BL/KsJ-*db/db* (*db/db*) mice, one of the most widely used animal models for obesity and type 2 diabetes. Our results suggest that AR inhibition prevents AOM-induced ACF formation by decreasing various inflammatory markers. Further, we also investigated in vitro the role of AR inhibition on various inflammatory markers in HG-induced HT29 human colon cancer cells. Our in vitro results also suggest that inhibition of AR prevents the expression of inflammatory markers in HT29 colon cancer cells.

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Materials and methods

Materials

4-week-old male C57BL/KsJ-*db/db* mice purchased from Jackson Laboratories (Bar Harbor, ME) were housed in pathogen-free conditions at the institutional animal care facility with free access to food and water. The animals were maintained in accordance to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and in accordance with the Institution's 'Guideline of the Animal Care and Use Committee'. Mice were kept in suspended cages ~10 cm above bedding trays with a 12 h light–dark cycle in the animal facility. Temperature and relative humidity were controlled at 21 °C and 55% respectively. AOM was bought from the Sigma-Aldrich Chemical Company (St. Louis, MO), and fidarestat was obtained as a gift chemical from Livwel Therapeutics, Inc. (USA). Antibodies against COX-2, iNOS, cyclin D1, survivin, XIAP, β-catenin and protein kinase C (PKC) β2, phospho-AKT, total and phospho-NF-κB P65, and GAPDH were obtained from Cell Signal, Inc. All other reagents used were of analytical grade.

AOM-induced colon carcinogenesis and ACF analysis

Approximately 6-week-old *db/db* mice were divided into three groups with six mice in each group. Mice in groups 2 and 3 were given AOM in sterile saline, at a dose of 10 mg/kg body wt intraperitoneally once a week, for 3 weeks. In group 3, mice were given AR inhibitor, fidarestat (50 mg/kg body wt, in drinking water) after 24 h of first AOM injection and continued for the entire period (10 weeks). Mice in group 1 received equal volume of sterile saline. All mice were euthanized by exposure to CO_2 followed by cervical dislocation. The colons were removed, flushed with saline and opened from anus to cecum and fixed flat between two pieces of filter paper in 10% buffered formalin for 24 h. Colons were stained with 0.2% methylene blue dissolved in saline, and the numbers of ACF were counted under the microscope.

Determination of cytokines/chemokines

The levels of cytokines and chemokines in the mice sera were determined by the Milliplex *MAG* mouse cytokine/chemokine magnetic bead array panel along with Luminex xMAP detection method as per manufacturer's protocol using a Millipore Multiplex system. The results are expressed as picograms per milliliter.

Immunohistochemistry

For subsequent microscopic evaluation of ACF, the colons were Swiss-rolled and embedded in paraffin. For immunohistochemical (IHC) analyses, serial sections (5 μm) of colon were cut as described before [19]. Briefly, slides were warmed at 60 °C for 1 h and deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. Antigen retrieval was performed by boiling slides in 10 mM sodium citrate (pH 6.0) for 10 min followed by blocking peroxidase reaction with 3% H_2O_2. Subsequently, the sections were rinsed in phosphate-buffered saline twice and incubated with blocking buffer (2% bovine serum albumin, 0.1% Triton X-100 and 2% normal goat serum) overnight at 4 °C. The sections were incubated with primary antibodies against proliferating cell nuclear antigen (PCNA), COX-2, AR, iNOS, cyclin D1, and phospho-NF-κB P65 for 1 h at room temperature. Antigen–antibody binding was detected by using DakoCytomation LSAB System-HRP kit. Sections were examined by bright-field light microscopy (EPI-800 microscope; Nikon, Tokyo, Japan) and photographed with a camera (Nikon) fitted to the microscope. Photomicrographs of the stained sections were acquired using an EPI-800 microscope (bright-field) connected to a Nikon camera.

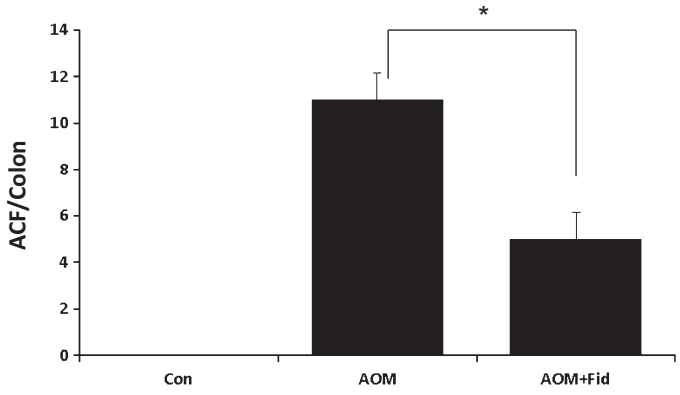

Fig. 1. Inhibition of AR prevents AOM-induced ACF formation in *db/db* mice. Mice were divided into three groups: (i) control; (ii) AOM (10 mg/kg body wt, weekly for 3 weeks) and (iii) received fidarestat (50 mg/kg body wt, in drinking water) throughout the study after 24 h of first AOM injection. Mice were euthanized 10 weeks after first AOM injection and colons were stained and examined for ACF formation. Data represent means ± SD (n = 6) from the various experimental groups. *P = 0.0026, AOM alone vs AOM + Fid. Con, control; AOM, azoxymethane; AOM + Fid, azoxymethane + fidarestat.

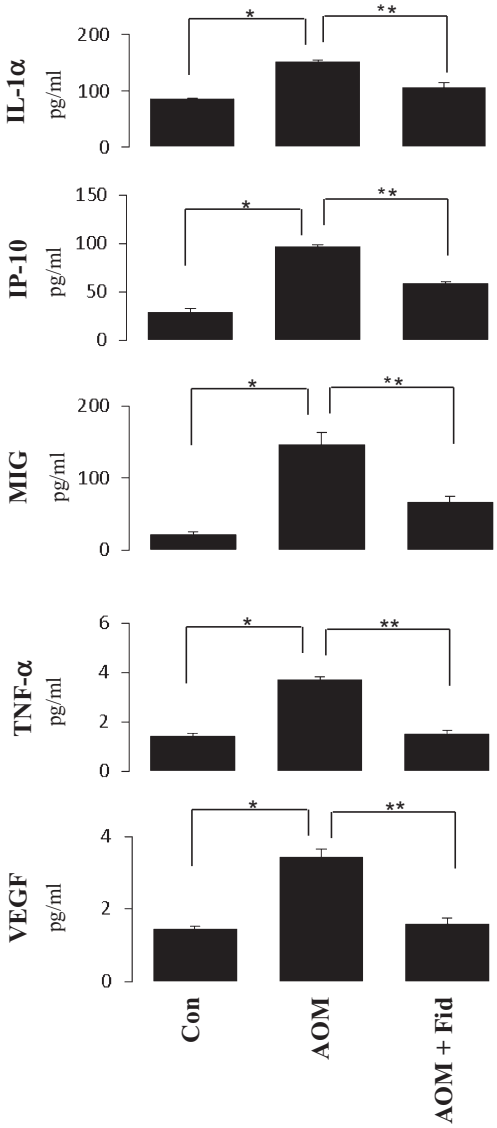

Fig. 2. Inhibition of AR suppressed AOM-induced increase in cytokines and chemokines in sera of *db/db* mice. Milliplex *MAG* mouse cytokine/chemokine magnetic bead panel along with Luminex xMAP detection method was used to determine cytokines and chemokines. Results are expressed as the mean ± SD (n = 6); *P < 0.05, Con vs AOM alone; **P < 0.05, AOM alone vs AOM + Fid. Con, control; AOM, azoxymethane; AOM + Fid, azoxymethane + fidarestat.

Western blot analysis

Colon extracts were prepared in radio immunoprecipitation assay (RIPA) cell lysis buffer and an equal amount of protein was separated on 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, electroblotted on nitrocellulose membranes and probed with specific antibodies against COX-2, iNOS, cyclin D1, survivin, β-catenin and protein kinase C (PKC) β2, phospho-AKT, total and phospho-NF-κB P65, and GAPDH. Antibody binding was detected by enhanced pico chemiluminescence (Pierce, Rockford, IL).