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Exenatide suppresses 1,2-dimethylhydrazine-induced colon cancer in diabetic mice: Effect on tumor angiogenesis and cell proliferation

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ABSTRACT

Colon cancer is the third leading cause of cancer mortality worldwide, which results from interactions of different factors. It is frequently a pathological consequence of persistent inflammation. Diabetes affects several cancers and is positively correlated with the incidence of colon cancer. This study aimed to study the effect of exenatide in ameliorating inflammation, angiogenesis and cell proliferation in 1,2-dimethyl hydrazine (DMH) induced colorectal carcinoma in diabetic mice. Mice were randomly allocated into six groups, 8 mice each. Group 1: vehicle control group. Group 2: diabetic control group. Group 3: DMH control group: diabetic mice treated with DMH (20 mg/kg/week, s.c.) for 15 week. Group 4: DMH-cisplatin group: mice received cisplatin (4 mg/kg/week, i.p.). Groups 5 & 6: DMH-exenatide (10 and 20 μg/kg) group: mice received exenatide (10 or 20 μg/kg/day, s.c.), respectively. The present results highlighted an increase in angiogenic markers and cell proliferation in the DMH-diabetic group in comparison with the control group with greater expression of endothelial marker (CD_{34}) and Ki-67 in colon tissue. Monotherapy with cisplatin or exenatide (10 and 20 μg/kg) downregulated these markers to different extents. The current results provided evidence that exenatide represents a promising chemopreventive effect against DMH-induced colon carcinogenesis in diabetic mice, at least in part, attributed to its anti-angiogenic and anti-proliferative mechanisms.

1. Introduction

Colorectal carcinoma (CRC) is a serious health problem representing the third most common cancer worldwide [1]. Among chemically-induced animal models of cancer, the 1,2-dimethylhydrazine (DMH) model is commonly used. Experimental colon cancer induced by DMH in rodents mimics histopathological and molecular characteristics of human colon cancer model, therefore it is considered as an ideal model for chemoprevention studies [2].

Colon cancer shares many of the risk factors with type 2 diabetes, in which hyperglycemia, hyperinsulinemia and insulin resistance facilitates neoplastic proliferation [3]. Inflammation with its consequent enhanced cell proliferation and angiogenesis are considered to be critical in the early events of CRC and represents a hallmark scenario in tumor initiation, promotion and progression [4,5]. Angiogenesis involves a sequence of events that is initiated by the increased expression of angiogenic factors as vascular endothelial growth factor (VEGF) and microvessel density [6]. In colon carcinoma cells, VEGF, an angiogenic factor that supports tumor growth production was increased [7]. As diabetes mellitus is closely correlated with an increased risk of CRC development and hyperglycemia facilitates neoplastic proliferation in diabetic subjects [4], the use of certain glucose-lowering medications on the basis of cancer concerns has been attracting attention and becomes a subject of intense investigation in diabetes therapy. There is considerable interest in understanding whether antidiabetic therapies impact cancer cell growth and influence the colon cancer behavior.

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by intestinal L-cells [8], stimulate insulin secretion and reduce glucagon secretion. Incretin or GLP-1-based therapy, involves using either a GLP-1 receptor agonist like exenatide or preventing the GLP-1 degradation by DPP-4 enzyme [9]. Incretin based therapies have extra-pancreatic effects that has recently aroused interest. Increasing evidences have suggested that GLP-1 receptors have been found in extra-pancreatic tissues, e.g., the central nervous system, the cardiovascular system and the gastrointestinal system [10]. GLP-1-related therapies has potent pleiotropic benefits, beyond their glycemic control which may

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provide additional benefits in the treatment of diabetes mellitus and its complications [11]. More recent evidence suggest that incretin-based therapeutic agents play a unique role in modulating the inflammatory process and endothelial function [12–14], in addition to its beneficial effects in inhibiting enhanced cell proliferation [11,14,15]. During the course of D.M., sustained compensatory GLP-1R activation and its consequence enhanced cell proliferation and inhibition of apoptosis are considered to be a critical pathophysiological sequences associated with CRC development. The use of GLP-1-based therapy may attenuate this compensation and reduce the risk of cancer development [16]. Therefore, if the use of GLP-1-based therapy in treatment of diabetes mellitus may influence the behavior of colon cancer will be of ongoing interest.

Hence, GLP-1-based therapy may be logic candidates in colon cancer studies associated with diabetes not only due to its potential role in diabetes therapy and its possible modifying role in cancer [11,14–16], but also because there is massive GLP-1R overexpression in selected gastrointestinal tumors, that is considered an important prerequisite for a successful targeting to peptide receptor [15].

On the basis of previous considerations, the present study was conducted to investigate the possible chemopreventive effect of exenatide against DMH-induced colon carcinogenesis in diabetic mice. This effect was determined in term of the anti-inflammatory, anti-angiogenic and anti-proliferative effect.

2. Materials and methods

2.1. Experimental animals

Forty eight male Swiss albino mice weighing 19–25 g were obtained from the Egyptian Organization for Biological Products and Vaccines (Vacsera, Egypt). Before experiments, the animals were allowed to adapt to the new environment for 2 weeks. Food and water ad libitum were allowed during the study period. Mice were housed under controlled conditioning (25 ± 3 °C and normal dark-light cycle). All the animal experiments were approved by the Institutional Animal Care and Use Committee at the Suez Canal University according to internationally accepted guidelines (NIH) Guide for the Care and Use of Laboratory Animals.

2.2. Drugs and chemicals

Streptozotocin (STZ) and DMH were purchased from Sigma–Aldrich (MO, USA). STZ was supplied in solid form and freshly prepared in 0.1 M citrate buffer solution (pH 4.5). DMH was supplied in concentrated solution and diluted with sterile saline. Exenatide injection (Bydureon vials, Astrea Zeneca, 2 mg/2 ml solution) was diluted with sterile saline. Cisplatin injection (Unistin vials, 10 mg/10 ml solution) was diluted with sterile saline.

2.3. Induction of type 2 diabetes and colonic cancer in mice

Mice were fed with a high-fat diet (HFD) for four weeks, prepared by mixing 20% sucrose (w/w) and 10% lard (w/w) into basal diet (BD). Then, mice were fasted overnight then received a single i.p. injection of STZ (30 mg/kg) [17] in citrate buffer in a volume of 16 ml/kg. One week later, fasting blood glucose was determined for each mouse using a blood sample taken from the tail vein using One Touch Ultra Mini glucometer (USA). Mice with fasting blood glucose level more than 200 mg/dl were considered diabetic and included in the experiment.

For induction of colonic cancer, mice received a single dose of DMH (20 mg/kg/week) by subcutaneous injection for 15 weeks [18]. Treatment with DMH started one week after STZ injection and continued for fifteen weeks.

2.4. Experimental groups

Mice were randomly allocated into six groups, 8 mice each. Group 1: vehicle control group: normal mice treated with saline (16 ml/kg/week s.c.) starting from the beginning of week 2 and continued for 15 weeks. Group 2: diabetic control group: diabetic mice treated with saline (16 ml/kg/week s.c.) starting from the beginning of week 2 and continued for 15 weeks. All the following groups are diabetic mice injected with DMH (20 mg/kg/week, s.c.) starting from week 2 and continued for 15 weeks. Group 3: DMH control group: diabetic mice treated with DMH and received a weekly injection of saline. Group 4: DMH-cisplatin treated group: mice received a weekly injection of cisplatin (4 mg/kg, i.p.). Group 5: DMH–Exenatide (10 μg/kg) treated group: mice received a daily dose of exenatide (10 μg/kg, s.c.) [19]. Group 6: DMH–exenatide (20 μg/kg) treated group: mice received a daily dose of exenatide (20 μg/kg, s.c.). Treatment with cisplatin and exenatide were scheduled to be injected weekly starting from the first week of DMH injection until the end of the experiment (end of week 16). In general, STZ and DMH (or saline) were injected in a volume equals 16 ml/kg.

2.5. Blood collection and serum separation

At the end of the experiment, mice were anaesthetized by using thiopental sodium (50 mg/kg, i.p.) [20] and killed by decapitation. Blood samples were collected by cardiac puncture in dry tubes and then centrifuged for 10 min to obtain the serum. After that, serum samples were separated and stored at −80 °C until use.

2.6. Determination of serum COX 2, IL-6 and VEGF by ELISA kits

Enzyme linked immunosorbent assay (ELISA) kits for cyclooxygenase 2 (COX 2; Glory Science Co., Ltd, TX, USA), IL-6 (Glory Science Co., Ltd, TX, USA), and VEGF (Biosource International Inc., CA, USA) were used to measure these parameters in serum samples. Methods were applied according to the manufacturer's instructions using an automated ELISA reader (Metertech, M960).

2.7. Histopathological examination of colon tissue

Tissues sections were stained with H&E, and then tumor cells in colon were evaluated and graded according to the degree of dysplasia of cells, hyperplasia and inflammatory reactions in the mucosal layer. 0 = free from dysplasia, hyperplasia or inflammatory reactions, 1 = no dysplasia, mild hyperplasia with or without mild inflammatory reaction presents, 2 = moderate hyperplasia with mild dysplasia with or without inflammatory reaction, 3 = marked hyperplasia with: mild to moderate dysplasia or inflammatory reaction or both, 4 = marked dysplastic and hyperplastic activity with inflammatory reaction with fibrosis and congestion [21].

2.8. Immunohistochemistry and image analysis

Tissue specimens from the colon of mice were dissected and fixed overnight in 10% paraformaldehyde solution and embedded in paraffin. Rabbit polyclonal antibodies against mice VEGF receptors 2, mice monoclonal antibodies against CD_{34} (Bio SB, Santa Barbara, California, USA) and rabbit polyclonal antibodies against Ki-67 (Abcam, Cambridge, UK) were used. Methods were applied according to the manufacturer's instructions.

Then slides were examined using light microscope (Olympus cx21, Japan). Immunopositive areas were manually outlined, then