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Melatonin suppresses AOM/DSS-induced large bowel oncogenesis in rats

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ABSTRACT

The inhibitory effects of exogenous melatonin (MEL) on colon oncogenesis were investigated using an azoxymethane (AOM)/dextran sodium sulfate (DSS) rat model. Male F344 rats initiated with a single intraperitoneal injection of AOM (20 mg/kg bw) were promoted by 1% (w/v) DSS in drinking water for 7 days. They were then given 0.4, 2 or 10 ppm MEL in drinking water for 17 weeks. At week 20, the development of colonic adenocarcinoma was significantly inhibited by the administration with MEL dose-dependently. MEL exposure modulated the mitotic and apoptotic indices in the colonic adenocarcinomas that developed and lowered the immunohistochemical expression of nuclear factor kappa B, tumor necrosis factor α, interleukin-1β and STAT3 in the epithelial malignancies. These results may indicate the beneficial effects of MEL on colitis-related colon carcinogenesis and a potential application for inhibiting colorectal cancer development in the inflamed colon.

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1. Introduction

The pineal production of melatonin (MEL) is controlled by an endogenous clock, the suprachiasmatic nuclei (SNC) of the hypothalamus, which is synchronized by the light/dark cycle, detected by photoreceptors located at the retinal ganglion cells. Neurons from the SCN project to the superior cervical ganglia and postganglionic noradrenergic fibers innervate pinealocytes. In darkness, the norepinephrine released from these sympathetic fibers promotes the synthesis of MEL. The maximum production of MEL within the pinealocytes occurs at night in response of a signal from the eye indication the absence of light. MEL acts as a circadian rhythm monitor, free radical scavenger and antioxidant, cytoprotective agent, immunomodulator, endocrine modulator, oncostatic agent and thermo regulator [1,2]. A pineal disturbance influences the pathogenesis and the phenotypic variations of metabolic syndrome [3]. Regarding the effects of MEL on oncogenesis, epidemiological studies provided evidence of the potential risk factor of alight at night in breast cancer with its involvement in the entire circadian axis rather than just MEL depression [4], endometrial cancer [5] and colorectal cancer (CRC) [6]. Experimental studies [7] suggest the protective effects of exogenous MEL on carcinogenesis mainly the in mammary gland [8] and other tissues including the colon [9–12], liver [13], skin [14] and pancreas [15]. The protective effects of MEL on oncogenesis are considered to be due to its antioxidative ability [16], antimutagenic potential [17] and alterations of MEL receptor-mediated metabolism [18]. Blask et al. postulated a new mechanism by which physiological and pharmacological blood levels of MEL inhibit cancer growth *in vivo* via a MEL-induced suppression of tumor linoleic acid uptake and its metabolism to the important mitogenic signaling molecule 13-hydroxyoctadecadienoic acid [19]. In addition, MEL is capable of inhibiting chemically induced colitis [20,21]. The anti-inflammatory action of MEL [22] is due to the suppression of COX-2 and iNOS expression [20] and the inhibition of nuclear factor-kappaB (NF-κB) [21]. These findings stimulate the clinical interest of MEL and suggest clinical applications of MEL and MEL agonists in oncology and chemoprevention [1].

Abbreviations: ALT, alanine aminotransferase; AOM, azoxymethane; AST, aspartate aminotransferase; COX, cyclooxygenase; CRC, colorectal cancer; DSS, dextran sodium sulfate; dUTP, deoxyuridine triphosphate; H & E, hematoxylin and eosin; HDL, high-density lipoprotein; IBD, inflammatory bowel disease; IL, interleukin; iNOS, inducible nitric oxide; LDL, low-density lipoprotein; MEL, melatonin; MI, mitotic index; NF-κB, nuclear factor-kappa B; PCNA, proliferative cell nuclear antigen; SNC, suprachiasmatic nuclei; ssDNA, single stranded DNA; T-Cho, total cholesterol; TdT, terminal deoxynucleotidyl transferase; TG, triglycerides; TNF, tumor necrosis factor; TUNEL, TdT-mediated dUTP-biotin nick end labeling; VLDL, very low-density lipoprotein.

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CRC is one of the leading causes of cancer deaths in the Western countries. Globally, the mortality of CRC is 655,000 deaths per year in 2005 [23]. Inflammation is known to be linked with CRC development as it is in epithelial malignancies in other tissues [24]. The risk of CRC in the patients with inflammatory bowel disease (IBD), including ulcerative colitis, increases with the increasing extent and duration of the disease [25]. A mouse model was recently established for colitis-related colon carcinogenesis [26] to investigate the pathogenesis [27–29] and chemoprevention [30,31] of inflammation-related CRC. In this mouse model of inflammation related two-stage colon carcinogenesis, different colonic carcinogens can be used in combination with a colitis-inducing agent, dextran sodium sulfate (DSS) and many colonic tumors develop within a short-term period [26,32–34]. In this model, the powerful tumor promoting effect of DSS may be due to oxidative/nitrosative stress caused by DSS-induced colitis [27–29]. This suggests that oxidative/nitrosative DNA damage associated with inflammation is involved in carcinogenesis and thus it is important to control the events that result in inflammation-related carcinogenesis [35]. In humans, oxidative stress also plays a key role in the pathogenesis of IBD-related intestinal damage [36].

Many drugs and chemopreventive agents are introduced for treatment or chemoprevention of IBD and IBD-related CRC [37]. The current study investigated whether MEL exerts cancer chemopreventive ability in colitis-associated colon carcinogenesis using a rat model [38,39], where the treatment schedule of AOM and DSS was similar to that in the mouse model [26]. In addition, the effects of MEL on the immunohistochemical expression of several biomarkers for colon oncogenesis including NF-κB, tumor necrosis factor (TNF)α, interleukin (IL)-1 and STAT3 [40,41] were studied in the colonic epithelial malignancies (adenocarcinomas). Additionally, effects of MEL on cell proliferation and apoptosis in the colonic adenocarcinomas were evaluated by the proliferation associated indices, proliferative cell nuclear antigen (PCNA) and Ki67 (MIB-5) and the apoptosis indices, the terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-biotin nick end labeling (TUNEL) method and the rabbit polyclonal anti-single stranded DNA (ssDNA) method.

2. Materials and methods

2.1. Animals, chemicals and diets

Male F344 rats (Charles River Japan, Tokyo, Japan) aged 5 weeks were used in this study. The animals were maintained in Kanazawa Medical University Animal Facility according to the Institutional Animal Care Guidelines. All animals were housed in plastic cages (3 or 4 rats/cage) with free access to tap water and a pelleted basal diet (CRF-1, Oriental Yeast, Co., Ltd., Tokyo, Japan) under controlled conditions of humidity (50 ± 10%), lightning (12-h light/dark cycle) and temperature (23 ± 2 °C). They were quarantined for 7 days after arrival and randomized by body weight into experimental and control groups. A colonic carcinogen AOM was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). DSS with a molecular weight of 36,000–50,000 was obtained from MP Biomedicals, LLC (Aurora, OH). DSS for induction of colitis was dissolved in distilled water at 1% (w/v). MEL (Sigma–Aldrich Chemical Co.) was dissolved in distilled water at concentrations of 0.4, 2 and 10 ppm (w/v) just before used. The preparation was done every day and animals received fresh MEL-containing drinking water in dark bottles. Animals had access to food and drinking water at all times. All handling and procedures were carried out in accordance with the Institutional Animal Care Guidelines.

2.2. Experimental procedures

The Institutional Animal Care and Use Committee evaluated all animal procedure associated with the present study and assured that all proposed methods were appropriate.

A total of 140 male F344 rats were divided into 9 experimental and control groups, as shown in Table 1. The rats in groups 1 through 6 were initiated by a single intraperitoneal injection of AOM (20 mg/kg body weight). Starting 1 week after the injection, 2% DSS in drinking water was administrated to rats of group 1 ($n = 25$) for 7 days and then followed without any further treatments for 18 weeks. Groups 2–4 ($n = 25$ for each group) were given drinking water containing 0.4, 2 and 10 ppm MEL for 17 weeks, respectively, starting 1 week after the cessation of DSS exposure. Group 5 ($n = 8$) received AOM and 10 ppm MEL. Group 6 ($n = 8$) was given AOM alone. Group 7 ($n = 8$) was given 2% DSS alone. Group 8 ($n = 8$) received 10 ppm MEL alone. Rats of group 9 ($n = 8$) did not receive any treatments and served as an untreated control. MEL was given to rats belonging to groups 2–5, 7 and 8 at night (from 18:00 to 9:00). The highest dose used in this experiment was based on the report by Li et al. [21], in which the dose significantly inhibited colitis-induced 2,4,6-trinitrobenzene by in rats. All animals were subjected to a complete gross necropsy examination at the time of euthanasia by CO_2 asphyxiation (week 20). The body, liver and spleen were weighed.

At necropsy, the colons were flushed with saline, excised, their length measured (from ileocecal junction to the anal verge), cut open longitudinally along the main axis and then washed with saline to remove feces. They were cut and fixed in 10% buffered formalin for at least 24 h. The histopathological examination was performed on paraffin-embedded sections, after staining with hematoxylin and eosin (H & E). Colonic tumors were diagnosed according to the Ward's description [42]. In brief, if the tumors with tubular formation invaded into the submucosa, the tumor was diagnosed as an adenocarcinoma. When the tumors with glandular structure did not invade the submucosa or depth and compressed the surrounding crypts, the tumor was diagnosed as an adenoma. The scoring (incidence and multiplicity) of the tumors was done on the H & E-stained tissue sections. The mitotic index (MI) was determined by counting number of mitoses per 100 adenocarcinoma cells on the H & E-stained sections.

2.3. Clinical chemistry

At the end of the 20-week experimental period, 5 rats randomly selected from each group were fasted overnight and then were anesthetized with sodium pentobarbital (30 mg/kg, i.p., Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) for a biochemical analysis. Blood from the inferior vena cava was collected into tubes containing EDTA and centrifuged (1500 × *g*, 10 min, 4 °C). The serum was aspirated and assayed as described below.

The serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed using commercially available kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum total cholesterol (T-Cho), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels were determined using commercial kits (BioVision Incorp., Mountain View, CA, USA). The serum glucose level was measured by the glucose oxidase method (Wako Pure Chemical Industries) and the serum levels of insulin (Wako Pure Chemical Industries) and leptin (GE Healthcare Bio-Sciences Corp., Picscataway, NJ, USA) were determined by sandwich ELISA kits.

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