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Ultraviolet A eye irradiation ameliorates colon carcinoma induced by azoxymethane and dextran sodium sulfate through β-endorphin and methionine-enkephalin

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Running title: The effect of UVA on colon carcinoma

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Abstract

Background: We previously reported that ultraviolet (UV) A eye irradiation reduces the ulcerative colitis induced by dextran sodium sulfate (DSS). This study examined the effects of UVA on colon carcinoma induced by azoxymethane (AOM) and DSS.

Methods: We irradiated the eyes of ICR mice with UVA at a dose of 110 kJ/m^2 using an FL20SBLB-A lamp for the experimental period.

Results: In mice treated with these drugs, the symptom of colon carcinoma were reduced by UVA eye irradiation. The levels of interleukin (IL)-6 and tumor necrosis factor (TNF)-α in the blood were increased in AOM+DSS-treated mice; however, those levels were reduced by UVA eye irradiation. The expression of β-endorphin, methionine-enkephalin (OGF), μ-opioid receptor, and opioid growth factor receptor (OGFR) of the colon were increased in the AOM+DSS-treated mice, and these levels were increased further following UVA eye irradiation. When β-endorphin inhibitor was administered, the ameliorative effect of UVA eye irradiation was reduced, and the effect of eye irradiation disappeared entirely following the administration of naltrexone (inhibitor of both opioid receptor and OGFR).

Conclusions: These results suggested that UVA eye irradiation exerts major effects on AOM+DSS-induced colon carcinoma.

Keywords: Ultraviolet A; AOM+DSS-induced colon carcinoma; β-endorphin; methionine-enkephalin; μ-opioid receptor; opioid growth factor receptor

Introduction

Ultraviolet (UV) is known to exert various influences on the human body. With respect to carcinoma, DNA damage is induced by UVB radiation, causing skin carcinoma (1). In addition, UV irradiation induces photo-immunosuppression, which results in the deterioration into melanoma (2). However, paradoxically, UVB irradiation also activates a vitamin D3 (3), which reduces the carcinogenic risk (4). In addition, a parallel relationship has been reported between the relief ratio of cancer and the amount of UV exposure from the sun (5). Various anti-carcinoma mechanisms of vitamin D have been reported. For example, the vitamin D3/vitamin D receptor obstructs the transcriptional activity of β-catenin against the Wnt/β-catenin signal transmission system activated by cancer cells, thereby exerting an anti-carcinoma effect (6). In addition, if vitamin D links with the vitamin D receptor combined with the cell membrane, proliferation signal transmission systems, such as phospholipase C, proteinkinase C, and phosphoinositide 3-kinase, will be activated, thereby reinforcing the differentiation induction or cell death induction of a cancer cells. (7). UV irradiation (UVR) therefore both induces and inhibits carcinoma.

Furthermore, UVR also exerts dynamic effects on the living body. Skin expresses neurohormones, the production and activities of which are regulated by UVR (8-14). In addition, the exposure of skin to UVB can activate the central hypothalamic-pituitary-adrenal axis and hypothalamus, which induces a rapid effect on immune activities through neural routing (15-17). Furthermore, UVR increases the expression of enkephalin, which is an endogeneous opioid (18). Thus, β-endorphin and enkephalin are induced by UVR and play the important roles in the immune system. Moreover, both are known to increase the risk of pancreatic cancer (19).

Colon carcinoma is one of the most frequent carcinomas, and its incidence is on the rise in Japan. Colon carcinoma is known to be strongly associated with lifestyle. In particular, with respect to diet, an abundant intake of fat and a deficiency in dietary fiber are causes (20,21). Drinking (22) and smoking (23) are also risk factors. Except the environmental factors, patients with familial adenomatous polyposis (24) or ulcerative colitis (25) often progress to colon carcinoma. Furthermore,

a hereditary predisposition has also been reported (26). Given the above, the risk of colon carcinoma is reduced by a healthy weight, moderate exercise, and good nutrition.

In our previous study, we reported that UVA eye irradiation ameliorated dextran sodium sulfate (DSS)-induced ulcerative colitis. However, the effect of UVA eye irradiation on colon carcinoma is not known. In this study, we examined the effect of UVA eye irradiation in a mouse model of colon carcinoma induced by azoxymethane (AOM) and DSS.

Materials and Methods

Animals

Specific-pathogen-free (SPF), 7-week-old female ICR mice (SLC, Hamamatsu, Shizuoka, Japan) were used in the experiments. The mice were kept individually in cages in an air-conditioned room at 23 ± 1°C, under controlled humidity (59% ± 10%) and light conditions (12-h light/dark cycle). There were six mice per group. This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of Suzuka University of Medical Science (approval number: 34). All surgeries were performed under pentobarbital anesthesia, and all efforts were made to minimize the animal's suffering.

Experimental design

The mice were quarantined for the first seven days and then randomly assigned to groups of six according to body weight as follows: control, UVA-irradiated, AOM+ DSS-treated, and AOM+DSS-treated UVA-irradiated (UVA/AOM+DSS-treated) groups. Mice in the AOM+DSS and UVA/AOM+DSS-treated groups received a single intraperitoneal injection of AOM (10 mg/kg body weight; Sigma Chemical Co., St. Louis, MO, USA); then, starting at 1 week after the injection, they received 2% DSS (molecular weight of 36000-50000; MP Biomedicals, Solon, OH, USA) in their drinking water for 1 week, with no further treatment for 18 weeks, as previously described (27). Control mice were untreated for the duration of the experiment. In the UVA-irradiated and UVA/AOM+DSS-treated groups, the eye was locally exposed to UVA irradiation (wavelength: 320-

400 nm; FL20SBLB-A lamp, Toshiba Co., Tokyo, Japan) 3 times a week for 18 weeks at a dose of 100 kJ/m^2 per day (irradiation time: 30 min/day) under light nembutal anesthesia. The rest of the body surface was protected from irradiation by aluminum foil. The procedure has been described in detail in previous studies (28,29). In the control experiments, the eye was exposed to normal visible light (wavelength: 400-700 nm; FL20SD light source; Toshiba Co.). With UVA exposure, the amelioration effect increases energy-dependently. The dose of 100 kJ/m^2 used here has been deemed the most effective amount of energy. No marked differences in the changes induced following UVA irradiation were noted among the eyes of the mice. We investigated the colon symptoms and extracted the colon and blood samples. The colon and blood samples were obtained 20 weeks after the start of the experiment.

Chemical treatment

N-(4-amino-2-methylquinolin-6-yl)-2-(4-ethylphenoxymethyl) benzamide monohydrochloride (JTC-801) treatment

JTC-801 (1 mg/kg/day; Selleck Chemicals, Houston, TX, USA), an antagonist of opioid receptor, was suspended in a 1:175 solution of DMSO:PBS, which was administered orally every other day over 18 weeks following DSS treatment (30).

Naltrexone treatment

Approximately 10 mg/kg of naltrexone (naltrexone hydrochloride, opioid and opioid growth factor receptor antagonist; Selleck Chemicals) in saline was injected intraperitoneally into the treatment mice every other day over 18 weeks following DSS treatment (31).

Preparation and staining of the colon

For the histological studies, the mice were sacrificed 20 weeks after the start of the experiment. The colon specimens were fixed in phosphate-buffered paraformaldehyde (4%), embedded in frozen

Tissue Tek, OCT compound, and cut into 5-μm-thick sections. The sections were then stained with hematoxylin-eosin (HE), in accordance with the established procedures, in order to enable the histological analysis of the colon. In addition, in colon specimens, apoptosis was analyzed using an in situ apoptosis detection kit (Takara Biomedicals, Tokyo, Japan).

Quantification of interleukin (IL)-6, tumor necrosis factor (TNF)-α, β-endorphin and methionine-enkephalin using an enzyme-linked immunosorbent assay (ELISA)

Blood and colon samples were taken at week 20 after the start of the experiment. The plasma levels of IL-6 and TNF-α and the colonal levels of β-endorphin and methionine-enkephalin were determined using commercial ELISA kits (IL-6: BioLegend, San Diego, CA, USA; TNF-α: R&D Systems, Minneapolis, MN, USA; β-endorphin: Phoenix Pharmaceutical, Belmont, CA, USA; methionine-enkephalin: Peninsula Laboratories International, Inc., San Carlos, CA, USA) in accordance with the manufacturer's instructions.

Western blotting analysis

The colon samples were homogenized in lysis buffer (Kurabo, Osaka, Japan), and centrifuged at 8000 g for 10 minutes. The supernatant from each samples was then isolated and stored at -80°C until the analysis. We performed a Western blotting analysis as previously described (32). Briefly, after the samples (protein: 10 μg/lane) were separated by electrophoresis, the membrane were incubated at 25°C for 1 h with primary antibodies against μ-opioid receptor (1:1000; Abcom, Tokyo, Japan), opioid growth factor receptor (OGFR, 1:1000; Mybiosource, San Diego, CA, USA), and Ki-67 (1:500; Santa Crus Biotechnology Inc., Santa Cruz, CA, USA) or β-actin (1:5000; Sigma-Aldrich, St. Louis, MO, USA). The membranes were then treated with a horseradish peroxidase-conjugated secondary antibody (1:1000; Novex, Frederick, MD, USA). The immune complexes were detected using ImmunoStar Zeta reagent (Wako, Osaka, Japan), and the images were acquired using the Multi-Gauge software program (Fujifilm, Greenwood, SC, USA).

Statistical analyses

All data were presented as mean ± standard deviation. Student's *t*-test was used to compare the means of the two groups, and differences were considered statistically significant at a p values under of <0.05.

Results

Effects of UVA eye irradiation on colon carcinoma induced by AOM+DSS treatment

After 20 weeks of treatment, blood and tissue samples were obtained, and the colon carcinomas were observed macroscopically. The symptoms of the colon carcinoma of UVA/AOM+DSS-treated mice were improved compared with AOM+DSS-treated mice (Figs. 1A and 1B). Using the blood samples, we evaluated the plasma IL-6 and TNF-α concentrations, as IL-6 and TNF-α are critical tumor promoters during early colitis-associated carcinogenesis (33). In the UVA/AOM+DSS-treated mice, the plasma levels of IL-6 and TNF-α were lower than in AOM+DSS-treated mice (Fig. 1C).

Effects of UVA eye irradiation on the levels of β-endorphin and methionine-enkephalin in the colon of AOM+DSS-treated mice

Increased expression of β-endorphin and methionine-enkephalin was observed in the AOM+DSS-treated mice. In the UVA/AOM+DSS-treated mice, the expression of β-endorphin and methionine-enkephalin was higher than in the AOM+DSS-treated mice (Fig. 2).

Effects of UVA eye irradiation on the expression of μ-opioid receptor and OGFR in the colon of AOM+DSS-treated mice

We then investigated the expression of μ-opioid receptor (receptor of β-endorphin) and OGFR (receptor of β-endorphin and methionine-enkephalin) in the colon. The expression of μ-opioid receptor and OGFR in the colon of UVA/AOM+DSS-treated mice was higher than in the AOM+DSS-treated mice (Fig. 3).

Effects of UVA eye irradiation on the expression of tunnel-positive cells and Ki-67 in the colon of AOM+DSS-treated mice

We also observed the expression of apoptosis and of Ki-67, which is a cell-proliferation marker. The proportion of tunnel-positive cells in the colon of UVA/AOM+DSS-treated mice was higher than in the AOM+DSS-treated mice (Fig. 4A). In contrast, the expression of Ki-67 in the colon of UVA/AOM+DSS-treated mice was lower than in the AOM+DSS-treated mice (Fig. 4B).

Effects of UVA eye irradiation on the AOM+DSS-treated mice after JTC-801 and naltrexone administration

In the JTC-801-treated UVA/AOM+DSS mice, the symptom of colon carcinoma were deteriorated compared with the untreated UVA/AOM+DSS mice. However, the symptom grades were milder than in the AOM+DSS-treated mice. With regard to the plasma IL-6 level, although JTC-801 treatment of UVA/AOM+DSS-treated mice reduction the level compared with AOM+DSS-treated mice, the value was higher than in the UVA/AOM+DSS-treated mice (Fig. 5A). In contrast, in the naltrexone-treated UVA/AOM+DSS mice, the number of lesions in the colon was not markedly different from that in the AOM+DSS-treated mice. In addition, the plasma levels of IL-6 were almost the same between the AOM+DSS-treated mice and the naltrexone-treated UVA/AOM+DSS mice (Fig. 5B)

Discussion

The present study demonstrated that UVA eye irradiation had ameliorative effects on AOM+DSS-induced colon carcinoma. The expression of β-endorphin and methionine-enkephalin in the colon of UVA/AOM+DSS-treated mice was higher than in the AOM+DSS-treated mice. Furthermore, the expression of μ-opioid receptor and OGFR in the colon of UVA/AOM+DSS-treated mice was higher than in the AOM+DSS-treated mice.

Hormones of POMC origin are known to be secreted following UVR skin exposure. In particular, β-endorphin, a hormone of POMC origin, stimulates the proliferation of epidermal kelatinocytes and plays a key role in the stress responses and immnunological modulation (34,35). In addition, opioids constitute a heterogeneous family of active peptides that play important roles in cutaneous nociception, immunomodulation, signal transduction and evoking or attenuating pruritus, depending on the differential receptor activation (36). We previously reported that UVA eye irradiation increased the β-endorphin secretion from the pituitary gland (37). Increases in the levels of β-endorphin and μ-opioid receptor, the receptor of β-endorphin, were also observed in this study (Fig. 2). As a narcotic drug, β-endorphin exerts analgesic action and induces euphoria through μ-opioid receptor within the brain (38). β-endorphin also strengthens immunity (39). Infections agent within the body have a receptor against a β-endorphin. If β-endorphin combines with this receptor, natural-killer cells, macrophages, and neutrophils are activated. These activated immune cells then attack the cancer cells and destroy them (40). In this study, the destruction of the cancer cells was observed as apoptosis (Fig. 4).

This activity of β-endorphin was inhibited by treatment with JTC-801, which is an inhibitor of μ-opioid receptor, and the ameliorative effect of UVA eye irradiation was subsequently reduced (Fig. 5). Of note, however: the ameliorative effects were not completely abolished by inhibition of the opioid receptor alone.

Methionine-enkephalin (a kind of opioid) has the inhibitive effect of cancer (41). In the present study, an increase in the expression of methionine-enkephalin was shown (Fig. 2). Methionine-enkephalin exerts its action by combining with OGFR, and an increase in the expression of OGFR

was also observed following UVA eye irradiation (Fig. 3). Although OGFR is a kind of opioid receptor, it has a different conformation from the usual opioid receptor. OGFR inhibits carcinoma cell growth via the following mechanism: when the cell cycle shifts to the S period from the G1 period, Rb protein of a cancer cell gene must be phosphorylated by the cyclin dependence kinase. After phosphorylation, Rb protein cannot combine with the transcription factor E2F, and free E2F promotes the expression of the gene relevant to cell proliferation, thereby continuing the cell cycle. However, the function of cyclin dependence kinase is strongly obstructed by cyclin dependence kinase inhibitor (p16, p21, et al.). By combining with OGF, OGFR enters the nucleus and increases the production of cyclin dependence kinase inhibitor. In this manner, methionine-enkephalin stops the cell cycle and prevents cell proliferation (42,43). In the present study, the levels of the cell proliferation marker Ki-67 were reduced in UVA/AOM+DSS-treated mice (Fig. 4). This decrease is thought to reflect the stagnation of the cell cycle by methionine-enkephalin. In addition, most of the ameliorative effects of UVA disappeared following the administration of naltrexone, which inhibits both opioid receptor and OGFR (Fig. 5). These findings demonstrated that the ameliorative effect of UVA was based on the action of both β-endorphin and methionine-enkephalin.

Conclusion

In the present study, we ameliorated AOM+DSS-induced colon carcinoma by UVA eye irradiation. Regarding the mechanisms, UVA eye irradiation induced increases in the expression of β-endorphin and methionine-enkephalin in the colon and in their respective receptors (Fig. 6). However, the mechanism of the signal transmission inducing these increases in the colon of UVA-irradiated mice is unknown. In addition, although we focused on opioids in this study, many different genes are affected by UVA eye irradiation. By analyzing the dynamic trends in UVA eye irradiation in detail, we may be able to develop new therapies for colon carcinoma.

Acknowledgments

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Figure legends

Fig. 1. The effects of UVA eye irradiation on AOM+DSS-induced colon carcinoma. (A) Macroscopic view and (B) hematoxylin and eosin staining of colon tissue sections after treatment. (C) Plasma IL-6 and (D) plasma TNF-α concentration in the colon carcinoma models. The values represent the mean ± SD derived from 6 animals. Student's *t*-test was used to compare groups. *, $P<0.05$. Scale bar = 100 μm.

Fig. 2. An analysis of the expression of β-endorphin and methionine-enkephalin in the colon. The values represent the mean ± SD derived from 6 animals. *, $P<0.05$.

Fig. 3. An analysis of the expression of μ-opioid receptor and OGFR in the colon. The values represent the mean ± SD derived from 6 animals. *, $P<0.05$.

Fig. 4. A Western blotting analysis of Ki-67 (A) and a histological analysis of apoptosis using a tunnel kit (B) in the colon. The values represent the mean ± SD derived from 6 animals. *, $P<0.05$. Scale bar = 100 μm.

Fig. 5. The effect of JTC-801 and naltrexone. (A) Macroscopic view of the colon and (B) plasma IL-6 and (C) plasma TNF-α concentrations in the colon carcinoma model. The values represent the mean ± SD derived from 6 animals. *, P<0.05.

Fig. 6. The mechanism of the effect of UV eye irradiation on AOM+DSS-induced colon carcinoma.

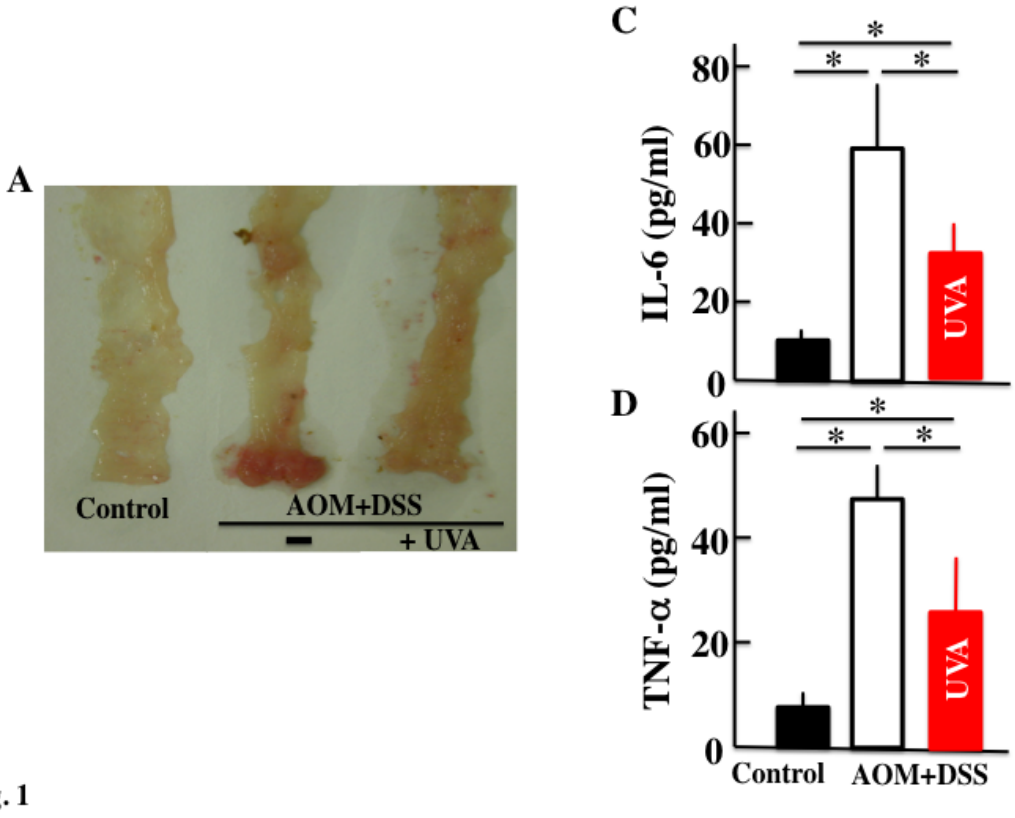
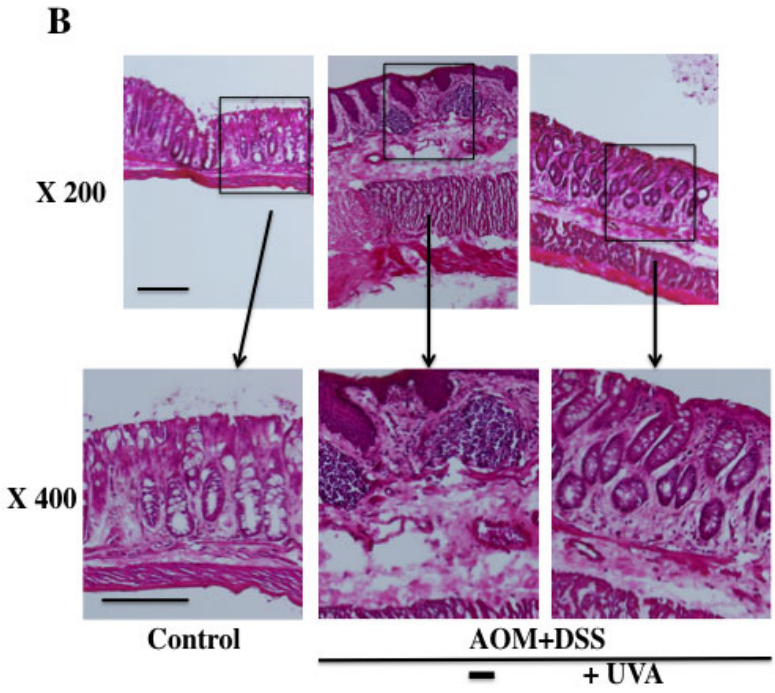


Fig. 1



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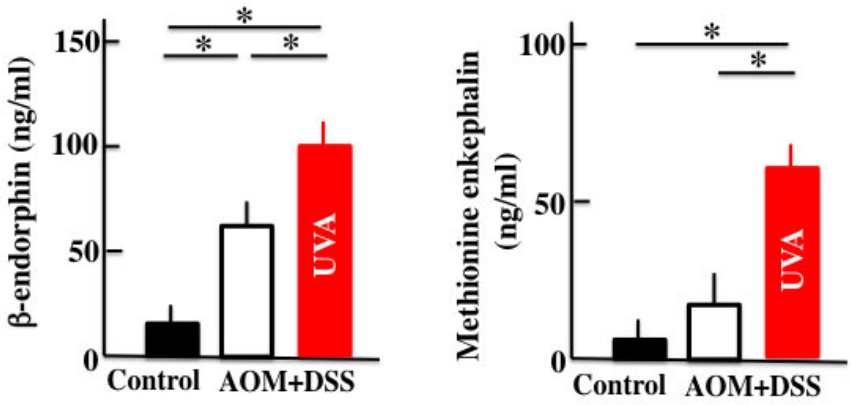
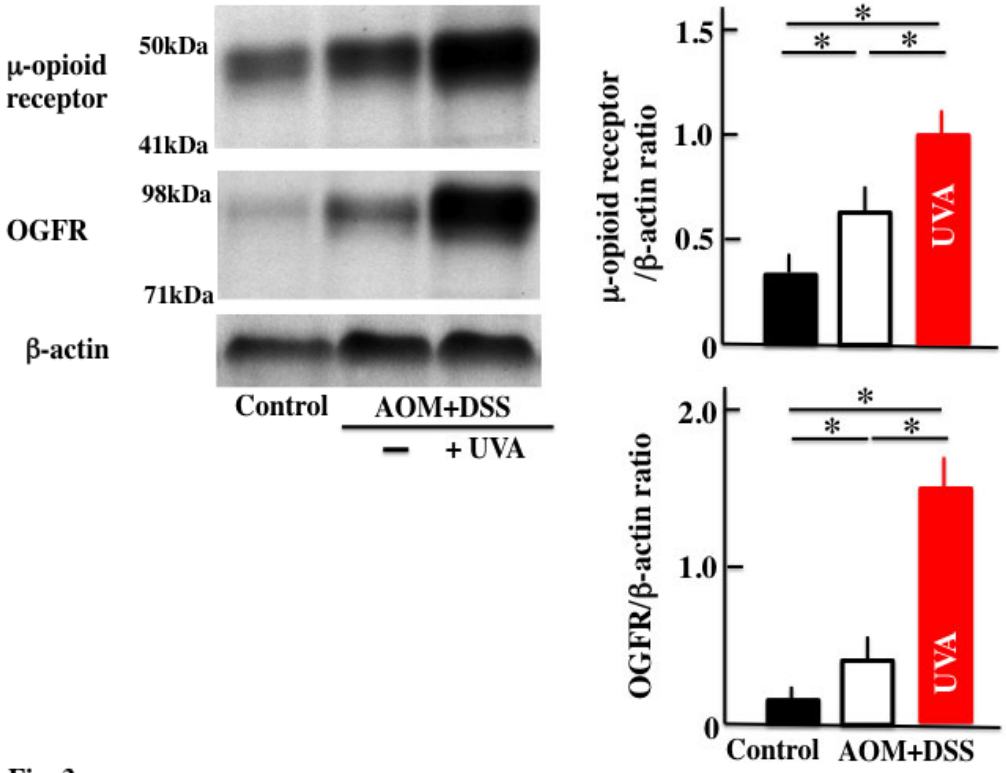


Fig. 2



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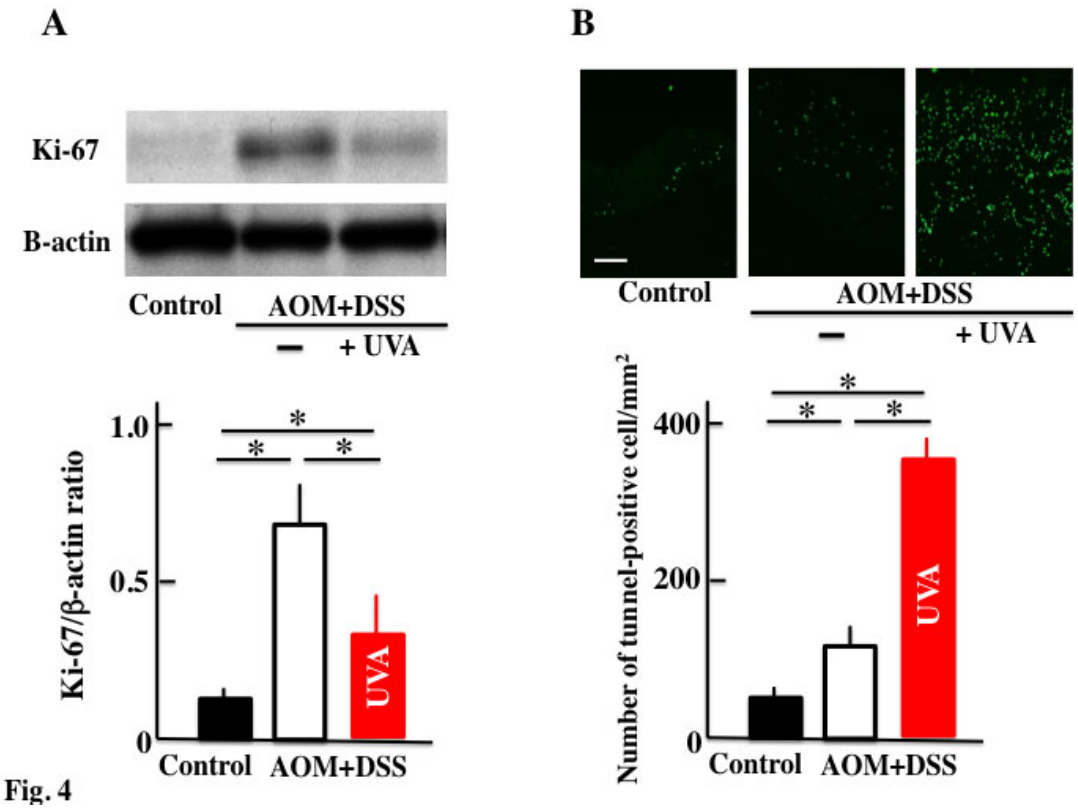

Fig. 4

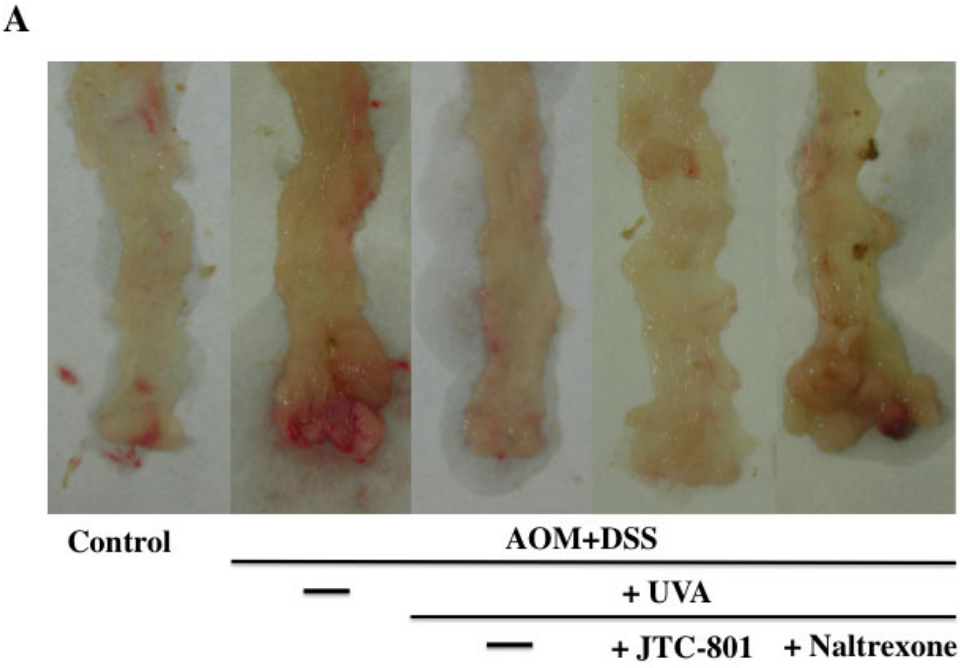

Fig. 5

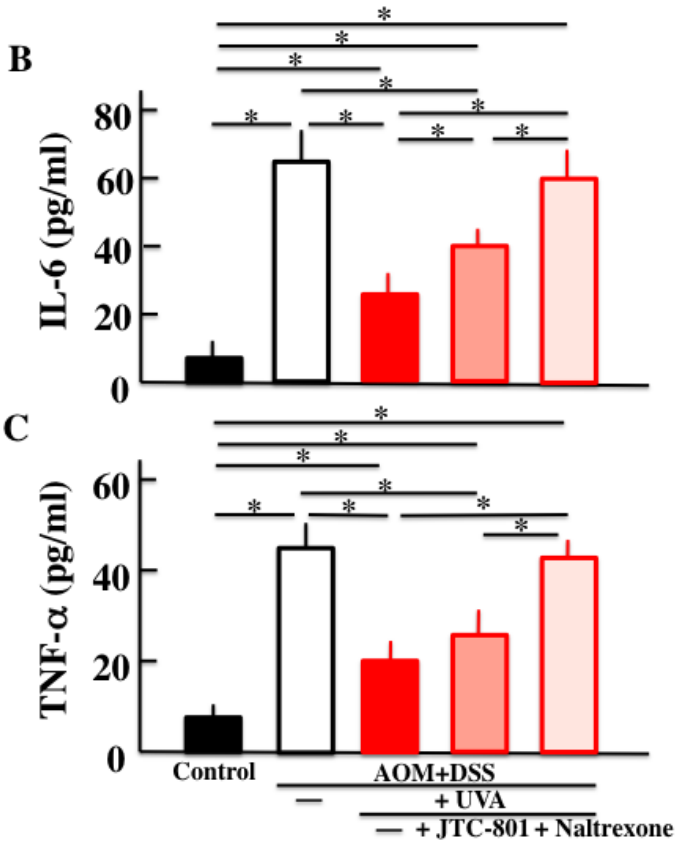

Fig. 5

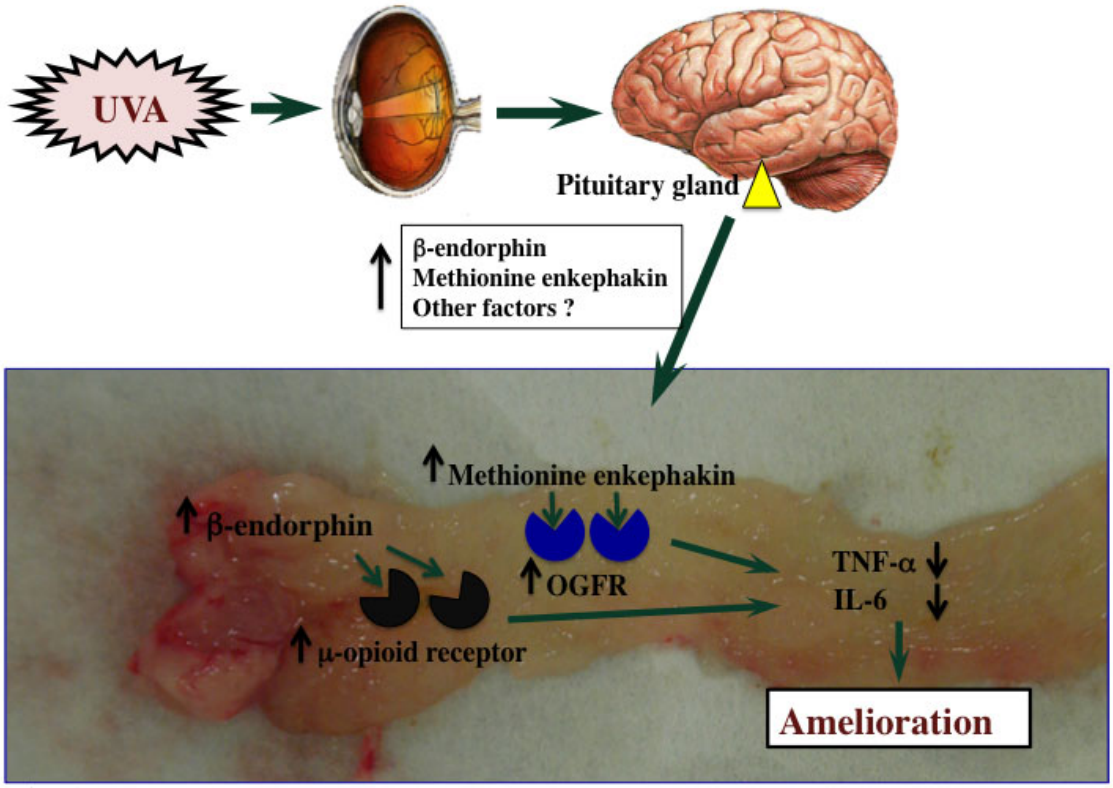

Fig. 6