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# The Effects of Ultraviolet Eye Irradiation on Dextran Sodium Sulfate-Induced Ulcerative Colitis in Mice

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## ABSTRACT

**Ultraviolet (UV) eye irradiation denatures the cells of the intestine. This study examined the action of UVA and UVB on dextran sodium sulfate (DSS)-induced ulcerative colitis. We produced a mouse model of ulcerative colitis by administering DSS for 5 days and irradiated the eye with UVB or UVA for each day of the DSS treatment period. DSS-induced ulcerative colitis was deteriorated by the UVB eye irradiation. Conversely, the symptoms improved with UVA eye irradiation. The levels of adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone (CRH), urocortin 2, interleukin (IL)-18, IL-6 and histamine in the blood increased after the UVB eye irradiation of DSS-treated mice (UVB/DSS-treated mice). In contrast, the β-endorphin level in the blood of the UVA/DSS-treated mice increased and the levels of urocortin 2, tumor necrosis factor (TNF)-α and histamine decreased. Furthermore, in the colon, the expression of melanocortin-2 receptors (MC2R) increased in the UVB/DSS-treated mice, while the expression of μ-opioid receptors increased in the UVA/DSS-treated mice. When an ACTH inhibitor was administered, UVB eye irradiation caused the deterioration of DSS-treated ulcerative colitis, while the effect of UV eye irradiation disappeared with a μ-opioid receptor antagonist. These results suggested that UV eye irradiation plays an important role in DSS-induced ulcerative colitis.**

## INTRODUCTION

Although there has been a rapid increase in the number of patients with ulcerative colitis in recent years, its cause remains unknown. However, many recent reports have investigated factors that cause the genesis of ulcerative colitis. The immune activation by bacteria and viruses, which leads to the invasion of the intestinal mucosa, causing lesions is a suggested etiology (1–5). The expression of inflammatory cytokines and the expression of adhesion molecules increase through this immune activation. As a result, this induces leukocyte invasion. Lesions of the intestinal mucosa are caused by the active oxygen produced in the leukocytes. The symptoms of ulcerative colitis develop when this series of responses occurs repeatedly. Ulcerative colitis may also be caused by irritation after exposure to an allergen (6–11). When the unusual activation of an immunological mechanism against a specific antigen takes place, chemical mediators, such as leukotriene B4, thromboxane A2, prostaglandin E2 and inflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α and IL-6, are released with a unique antigen, and these cause the inflammation of the intestinal mucosa. Other causes include psychosomatic disease and a physical stress (12–15). In a murine model subjected to restraint stress and oxidative stress, the increased invasion of leukocytes of the intestine and the permeable sthenia of the large intestine tunica mucosa were reported to be accompanied by the sthenia of interferon (IFN)-γ and other factors.

It is known that UV will induce pigmentation (16), inflammation (17), immunosuppression (18) and cancer (19) of the skin. Moreover, when the eye is subjected to UV irradiation, it also affects sites that are not directly exposed to UV, such as an intestine (20) and ovaries (21). Furthermore, UV eye irradiation gas caused the systemic fatigue and physical stress (22). However, there have been no reports about the influence of UV eye irradiation on ulcerative colitis.

In this study, we observed the influence of UV eye irradiation in a mouse model of dextran sodium sulfate (DSS)-induced ulcerative colitis.

## MATERIALS AND METHODS

*Animal experiments.* Specific pathogen-free, 8-week-old male C57BL/6j 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 SPF conditions. In six mice per group, we investigated the colon symptoms and extracted the colon and blood samples. The colon and blood samples were obtained 5 days after the start of the experiment. 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 suffering. The eye was locally exposed to UVB (wavelength 280–320 nm; 20SE sunlamp, Toshiba Co., Tokyo, Japan) or UVA (wavelength of 320–400 nm; L20S BLB-A lamp, Toshiba Co., Tokyo, Japan) during the experimental period at a dose of 1.0 kJ $m^{-2}$ $day^{-1}$ (UVB; irradiation time: 60 s $day^{-1}$), or 100 kJ $m^{-2}$ $day^{-1}$ (UVA; irradiation time: 30 min $day^{-1}$), respectively, with the animals kept 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 (20,23). In the control experiments, the eye was irradiated with visible light (wavelength: 400–700 nm; FL20SD light source, Toshiba Co., Tokyo, Japan). On UVA exposure, the amelioration effect increases to energy dependence and decreases bordering on 150 kJ $m^{-2}$. The 100 kJ $m^{-2}$ used here is the most effective amount of energy. On UVB exposure, the deterioration effect increases to energy dependence, and then, the grade of the

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deterioration effect becomes fixed, with a peak of 1.0 kJ $m^{-2}$. Notably, 100 kJ $m^{-2}$ (UVA) and 1.0 kJ $m^{-2}$ (UVB), which were used in the present examination, are the typical 1-h daytime doses received in Osaka, Japan. In addition, no marked differences in the changes induced following radiation exposure were noted between UVA and UVB radiation in the eyes of the mice.

*The induction of DSS-induced experimental ulcerative colitis in mice.* We divided 8-week-old C57BL/6j mice into six groups ($n = 6$ per group), which were subjected to UVA or UVB irradiation. The mice were fed 5.0% (W/V) DSS (molecular weight: 36 000–50 000 Da; MP Biomedicals, Solon, OH) via their drinking water to induce colitis (for 5 consecutive days). The development of colitis was monitored in each mouse by measuring their weight and observing the condition of their feces. The severity of colitis was determined based on their body weight, the condition of their feces and their colon length. The fecal condition score was determined using two parameters: stool consistency (0 = normal; 1 = soft; 2 = very soft, but formed; 3 = liquid) and fecal bleeding (0 = negative; 1 = faintly blue; 2 = moderately blue; 3 = dark blue; 4 = blood visible using the guaiac paper test); the sum was considered to be the individual's disease activity score (24).

*N-(4-amino-2-methylquinolin-6-yl)-2-(4-ethylphenoxymethy)benzamide monohydrochloride (JTC-801) treatment.* JTC-801 (1 mg $kg^{-1}$ $day^{-1}$; Selleck Chemicals, Hauston, TX), an opioid receptor antagonist, was suspended in a 1:175 solution of DMSO:PBS, which was administered orally on each day of the experiment (25).

*11β,17β -11-[4-(dimethylamino)-phenyl]-17-hydroxy-17-(1-propynyl) estra-4,9,-dien-3-one (RH-486) treatment.* RU-486 (30 mg $kg^{-1}$ $day^{-1}$; mifepristone, Roussel-Uclaf, Paris, France), a glucocorticoid receptor antagonist, was suspended in corn oil, which was intraperitoneally injected on each day of the experiment (26).

*The preparation and staining of the colon.* For the histological studies, the mice were sacrificed 5 days 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 (H&E), according to established procedures, to enable the histological analysis of the tissue.

*The quantification of adrenocorticotropic hormone, corticotropin-releasing hormone, β-endorphin, urocortin 2, interleukin (IL)-18, IL-6, tumor necrosis factor-α, histamine and substance P using an enzyme-linked immunosorbent assay.* Blood samples were taken from the heart on the fifth day after the start of the experiment. The plasma levels of adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone (CRH), β-endorphin, urocortin 2, IL-18, IL-6, tumor necrosis factor (TNF)-α, histamine and substance P were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (ACTH and β-endorphin: Phoenix Pharmaceuticals Inc., Burlingame, CA; CRH and urocortin 2: Yanaihara Lab., Shizuoka, Japan; IL-18: MBL., Nagoya, Japan; IL-6: BioLegend, San Diego, CA; TNF-α: R&D Systems, Minneapolis, MU; Histamine: Bertin Pharma, Montigny-le-Bretonneux, France; Substance P: Enzo Life Sciences Inc., Farmingdale, NY) according to the respective manufacturer's instructions. The optical density was measured with a microplate reader (Molecular Devices, Sunnyvale, CA).

*Western blotting analysis.* The colon samples were homogenized in lysis buffer (Kurabo, Osaka, Japan) and centrifuged at 8000 ***g*** for 10 min. 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 (27). Briefly, after the samples were separated by electrophoresis, the membranes were incubated at 25°C for 1 h with primary antibodies against melanocortin receptor-2 (MC2R) (1:1000; Millipore-Chemicon, Heule, Belgium), μ-opioid receptor (1:1000; Abcam, Tokyo, Japan), corticotropin-releasing hormone receptor (CRHR) type 1 (1:1000; GeneTex Inc., Irvine, CA, USA) and CRHR type 2 (1:1000; Novus Biologicals, Littleton, CO, USA) or β-actin (1:5000; Sigma-Aldrich, St. Louis, MO, USA). The membranes were then treated with a horseradish peroxidase-conjugated secondary antibody (Novex, Frederick, MD, USA). The immune complexes were detected using ImmunoStar Zeta regent (Wako, Osaka, Japan), and the images were acquired using the Multi-Gauge software program (Fujifilm, Greenwood, SC, USA).

*Statistical analysis.* The data are presented as the means ± standard deviation (Figs. 1C,G, 2, 3, 4C,F). The values indicate the mean and pooled standard error of the mean (Figs. 1A,B,E,F and 4A,B,D,E). Student's *t*-test or a one-way analysis of variance (ANOVA) was applied, as appropriate, for the comparisons among the test groups. *P* values of $<0.05$ were considered to indicate a statistically significant difference.

## RESULTS

### The effects of UV eye irradiation on the DSS-treated mice

Diarrhea and fecal bleeding were observed after the DSS treatment in the UVB/DSS- and UVA/DSS-treated mice. The disease activity score of the UVB/DSS-treated mice was higher than that of the DSS-treated mice, whereas the score of the UVA/DSS-treated mice was lower than that of the DSS-treated mice (Fig. 1A,D). DSS treatment also resulted in a dramatic decrease in body weight and colon length. The colon length of the UVB/DSS-treated mice was shorter than that of the DSS-treated mice, while the colon of the UVA/DSS-treated mice was longer than that of the DSS-treated mice (Fig. 1B,E). H&E staining also highlighted the cellular destruction of the intestinal epithelium in the colon of DSS-treated mice as well as edema in the submucosa. The colon of the UVA/DSS-treated mice was thinner than that of the DSS-treated mice. Moreover, in the colon of DSS-treated mice, the extensive decomposition of the tunica mucosa, edema of the tela submucosa, the formation of proud flesh and ulceration were observed. However, edema and ulcus were not observed, despite the observation of apoplexy in the UVA/DSS-treated mice. In addition, no differences of opinion were observed regarding the UVA-treated mice, while the colon damage of the UVB/DSS-treated mice was more severe than that of the DSS-treated mice (Fig. 1D,H).

### The effects of UVA eye irradiation on the plasma levels of ACTH, β-endorphin, CRH, urocortin 2, IL-18, IL-6, TNF-α, histamine and substance P in DSS-treated mice

Increased plasma concentrations of ACTH, β-endorphin, CRH, urocortin 2, TNF-α, histamine and substance P were observed in the DSS-treated mice after 5 days of treatment. In the UVB/DSS-treated mice, the plasma levels of ACTH, urocortin 2, IL-18, IL-6, CRH, histamine and substance P increased in comparison with those in the DSS-treated mice, while the levels of β-endorphin and TNF-α did not change. On the other hand, in the UVA/DSS-treated mice, the plasma level of β-endorphin increased in comparison with that in the DSS-treated mice, while the levels of urocortin 2, TNF-α, histamine and substance P decreased. There were no changes in the levels of ACTH, CRH, IL-18 and IL-6 (Fig. 2).

### The effects of UV eye irradiation on the expression of MC2R, μ-opioid receptor, CRHR type 1 and CRHR type 2 in the colon of DSS-treated mice

Next, we investigated the expression of MC2R (a receptor of ACTH), μ-opioid receptor (a β-endorphin receptor), CRHR type 1 (mainly a receptor of CRH) and CRHR type 2 (mainly a receptor of urocortin 2) in the colon. The expression of MC2R,

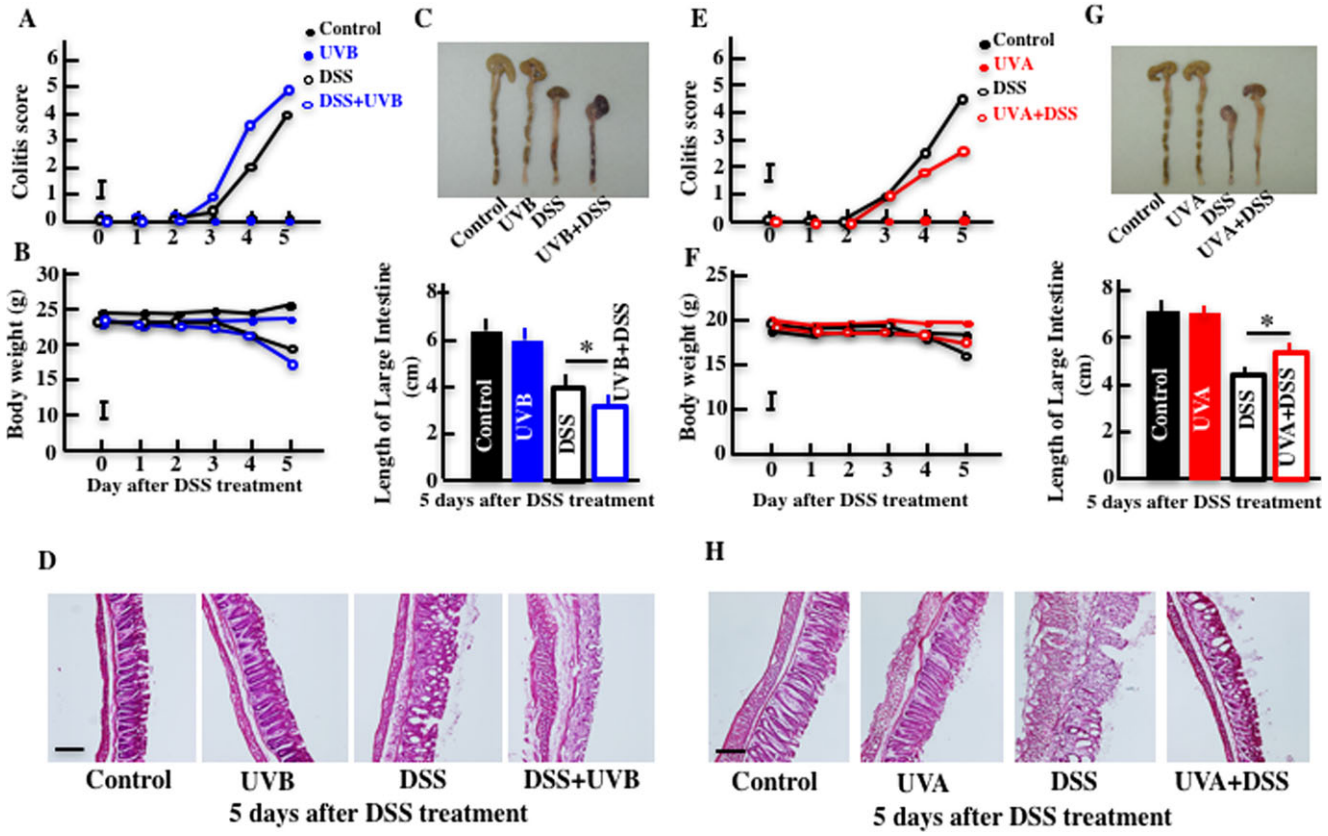


**Figure 1.** The effects of UVA and UVB eye irradiation on DSS-induced ulcerative colitis. The temporal response of the colitis score (A, E) and body weight (B, F). The length of the large intestine (C, G). A histological analysis of colon sections (D, H). Treatment groups ($n$ = 6 per group); control (untreated) and DSS (treated with 5.0% DSS for 5 consecutive days). Scale bar = 100 μm. The values represent the means ± SD derived from 6 animals (C, G). The values represent the mean and pooled SEM (A, B, E, F). *$P$ < 0.05 (DSS *vs* UVB + DSS and DSS *vs* UVA + DSS).

CRHR type 1 and CRHR type 2 in the colon of UVB/DSS-treated mice increased in comparison with that in the DSS-treated mice. In contrast, the expression of MC2R, CRHR type 1 and CRHR type 2 in the colon of the UVA/DSS-treated mice did not differ markedly from that in the DSS-treated mice (Fig. 3). On the other hand, although μ-opioid receptor expression in the colon of UVB/DSS-treated mice did not differ from that in the DSS-treated mice, its expression was increased in the UVA/DSS-treated mice in comparison with the DSS-treated mice (Fig. 3).

### The effects of UV eye irradiation on the DSS-treated mice after JTC-801 and RU486 injection

In the RU-486-treated mice, the decrease in body weight, the increase in colitis score and the shortening of the colon length improved after UVB/DSS treatment, with each of the values approaching the values of the control mice (Fig. 4A–C). Furthermore, although the weight, colitis score and the length of the colon improved after JTC-801 treatment in comparison with the UVB/DSS-treated mice, the values did not differ from those in the DSS-treated mice (Fig. 4A–C). In contrast, after JTC-801 treatment, the improvements to the length of the colon, the colitis score and the body weight that were observed after UVA/DSS treatment decreased to the point that they were not significantly different from those in DSS-treated mice (Fig. 4D–F). Furthermore, the body weight, the colitis score and colon length improved to a greater extent in the RU486-treated mice than they did in the UVA/DSS-treated mice (Fig. 4D–F).

## DISCUSSION

The present study demonstrated that UVB eye irradiation causes the deterioration of DSS-induced ulcerative colitis, while UVA eye irradiation leads to its improvement. Moreover, the levels of ACTH and urocortin 2 in the blood of UVB/DSS-treated mice increased in comparison with the levels of the DSS-treated mice, while there was an increase in the β-endorphin level in the blood of the DSS-treated mice. In addition, the expression of MC2R in the colon of the UVB/DSS-treated mice increased in comparison with that in the DSS-treated mice, while the expression of μ-opioid receptor increased in the UVA/DSS-treated mice.

The level of ACTH in the blood is increased by UV irradiation and physical stress. In the present study, both UVA and UVB eye irradiation were found to cause a similar increase in ACTH (Fig. 2). We previously showed that the increase in ACTH in the blood in response to UV eye irradiation is of the blood hypothalamo–pituitary–pro-opiomelanocortin pathway origin (28,29). The ACTH level in the blood of UVB/DSS-treated mice was remarkably increased in comparison with the DSS-treated and UVA/DSS-treated mice (Fig. 2). In a mouse model of restrain stress, Sekiyama *et al.* reported that IL-18 increased in the plasma and that the increase was obstructed by the anti-ACTH antibody (30). This indicates that ACTH released from

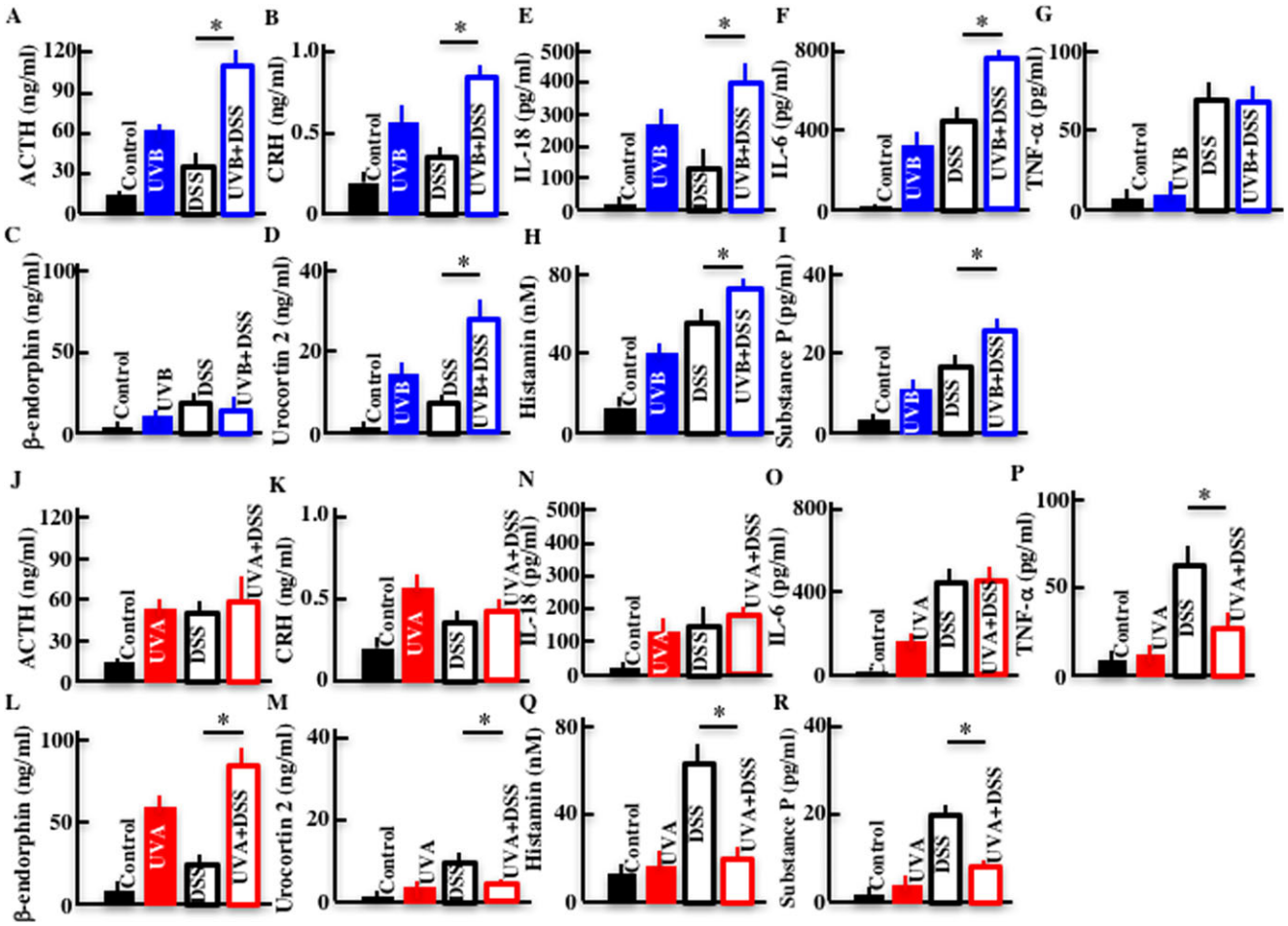


**Figure 2.** An analysis of the plasma ACTH (A, J), plasma CRH (B, K), plasma β-endorphin (C, L), plasma urocortin 2 (D, M), plasma IL-18 (E, N), plasma IL-6 (F, O), plasma TNF-α (G, P), plasma histamine (H, Q) and plasma substance P concentrations (I, R) after UVA/DSS or UVB/DSS treatment. The values represent the mean ± SD derived from 6 animals. *$P < 0.05$ (DSS *vs* UVB + DSS and DSS *vs* UVA + DSS).

the pituitary gland system leads to the activation of IL-18 in the adrenal gland through the hypothalamic–pituitary–adrenal axis. The activated IL-18 builds the cascade, which causes the fluctuation of various types of cytokines (including IL-6) and adhesion molecules further downstream, resulting in autoimmune lesions in the gut. In the present study, the IL-6 and IL-18 levels increased in the UVB/DSS-treated mice in comparison with those in the DSS-treated mice (Fig. 2), and the deterioration of ulcerative colitis was improved by the administration of an inhibitor of MC2R, which is a receptor of ACTH (Fig. 4). This shows that ACTH is one cause of the exacerbation of ulcerative colitis that occurs in response to UVB eye irradiation. As ulcerative colitis was ameliorated by the administration of RU486 in the UVB/DSS-treated mice, ACTH can be considered to have induced the adverse reaction. On the other hand, the deterioration of ulcerative colitis in response to UVB exposure was ameliorated by the administration of JTC-801, with the grade becoming equivalent to that in DSS-treated mice. With regard to the above-mentioned result, although it is thought that ulcerative colitis deteriorates to an equal or greater degree than that observed in UVB/DSS-treated mice due to the inhibition of the μ-opioid receptors, it was though that the level of β-endorphin in the blood increased by the inhibition of the μ-opioid receptors. The proposed feedback mechanism is that an increase in the level of the β-endorphin level causes a decrease in the production of β-endorphins and ACTH from POMC, resulting in a decreased grade of deterioration in comparison with UVB/DSS-treated mice. However, further studies will be required to elucidate the precise details of this mechanism.

On the other hand, the DSS-inducible ulcerative colitis was found to improve after UVA eye irradiation. Although the level of ACTH in the blood of the UVA/DSS-treated mice did not change in comparison with that in the DSS-treated mice, there was a remarkable increase in the β-endorphin level. β-Endorphin inhibits physical stress (31,32) and suppresses the increase in ACTH (33). Furthermore, β-endorphin suppresses the production of substance P (34), a neurotransmitter that is induced by pain. The increased production of substance P also increases histamine secretion. By suppressing substance P, β-endorphin may cause histamine disengagement and, as a result, may suppress the deterioration of ulcerative colitis. In contrast, the secretion of substance P was increased by the administration of a histamine, and the secretions of histamine and substance P both increase further over the course of the mutual transaction (35). In this study, the levels of substance P were found to increase (Fig. 2). From this, we may surmise that substance P may also be a cause of the adverse effects of UVB radiation. However, no reports have described any direct influence of UVB eye irradiation on levels

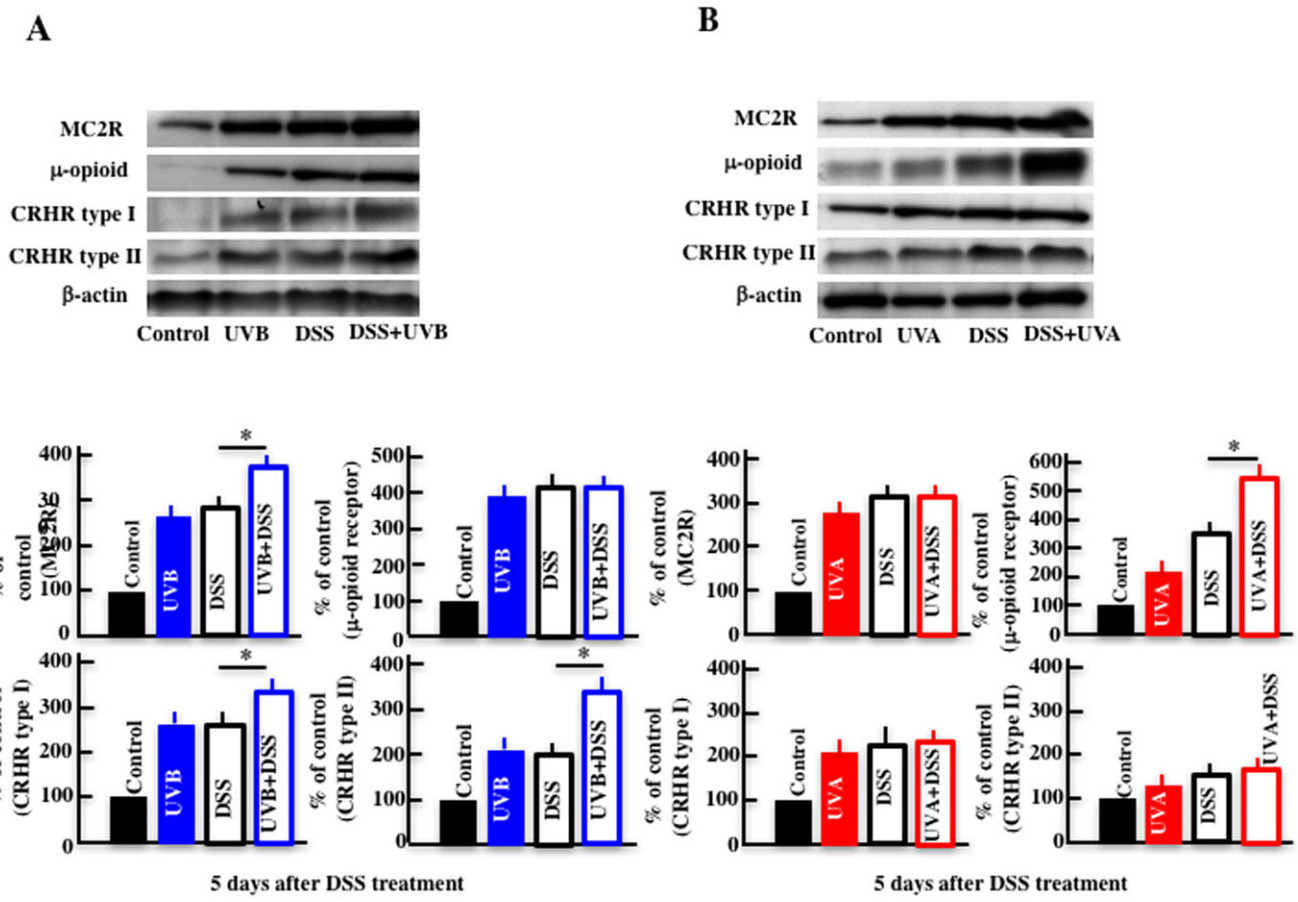


**Figure 3.** A Western blotting analysis of MC2R, μ-opioid receptors, CRHR type 1 and CRHR type 2 in the colon. (A) The UVB/DSS-treated group. (B) The UVA/DSS-treated group. The values represent the means ± SD derived from 6 animals. *$P < 0.05$ (DSS *vs* UVB + DSS and DSS *vs* UVA + DSS).

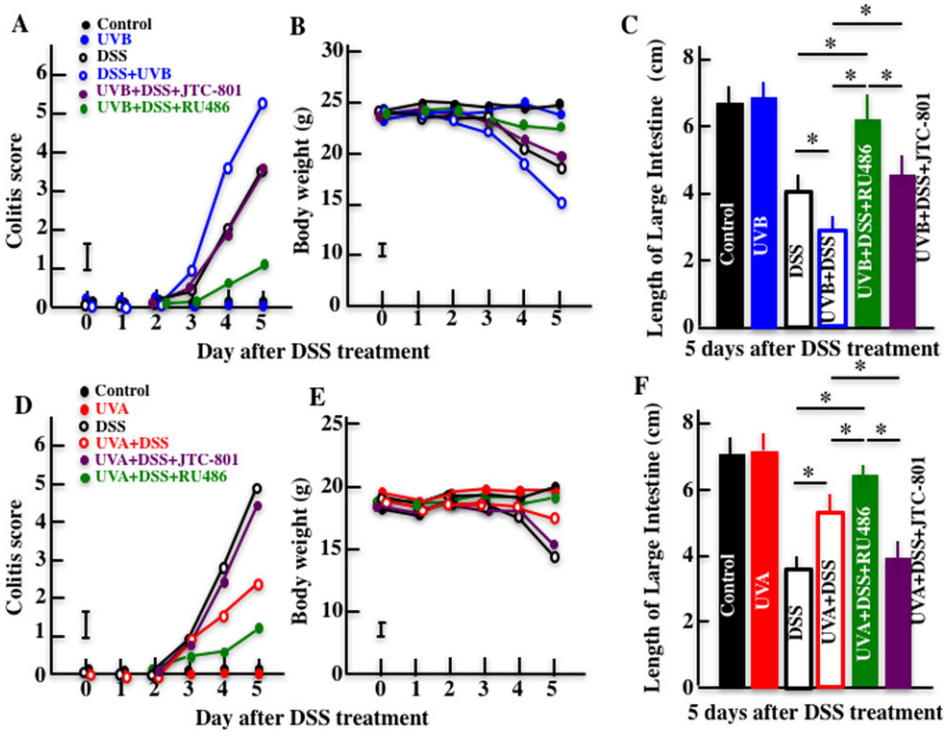


**Figure 4.** The effects of JTC-801 and RU486 on the colitis score, body weight and length of the large intestine of the UVB/DSS-treated (A, B, C) and UVA/DSS-treated mice (D, E, F). The values represent the means ± SD (C, F) and pooled SEM (A, B, D, E). *$P < 0.05$.

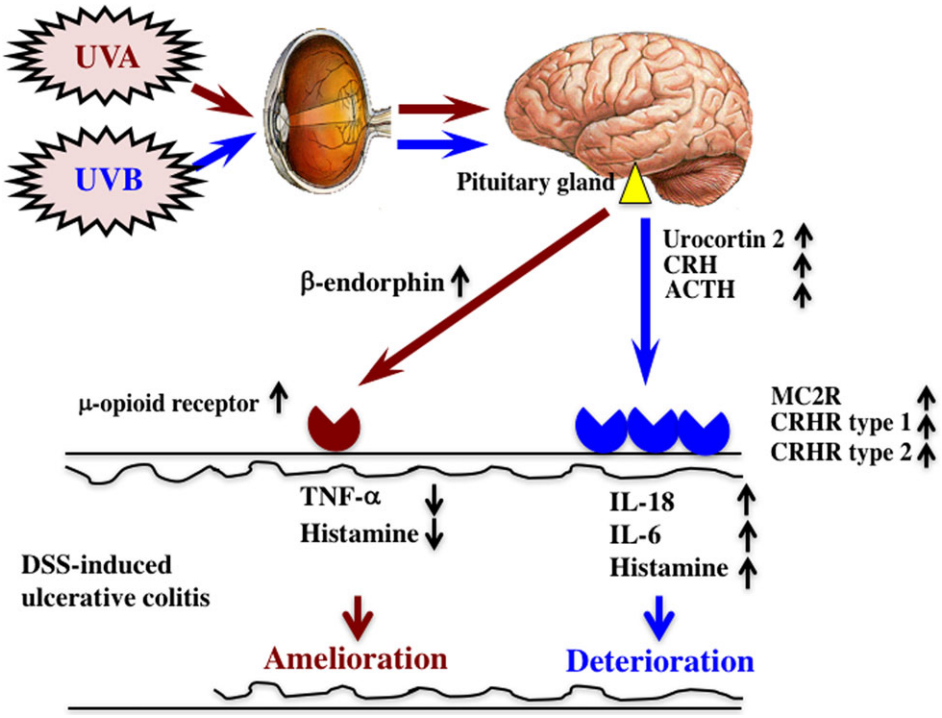


**Figure 5.** The mechanism of the effect of UV eye irradiation on DSS-induced ulcerative colitis.

of substance P. Substance P likely increased with increasing levels of histamine, which performed degranulation with urocortin 2. Furthermore, we found that after the administration of a μ-opioid receptor (a β-endorphin receptor) inhibitor, the symptoms of DSS-induced ulcerative colitis in the UVA/DSS-treated mice did not differ from those in DSS-treated mice (Fig. 4). Although the β-endorphin levels indicated an ameliorative effect against DSS-treated ulcerative colitis, the detailed mechanism underlying this effect remains to be elucidated. However, although the mechanism was examined using RU486 and JTC-801, this mechanism cannot be wholly elucidated using inhibitors alone. We must therefore conduct further experiments using an excessive expression of gene and knockout mouse models.

Furthermore, in the present study we observed changes in the blood levels of CRH and urocortin 2. The levels of urocortin 2 and CRH were observed to decrease in UVA/DSS-treated mice in comparison with DSS-treated mice, while both levels increased in UVB/DSS-treated mice (Fig. 2). Urocortin 2 combines with CRHR type 2 to promote the disengagement of histamines from mast cells (36). On the other hand, CRH combines with CRHR type 1 and increases the expression of ACTH (36). In present study, increases in the urocortin 2 and the histamine levels were induced in the blood of UVB/DSS-treated mice. Furthermore, the blood levels of CRH and ACTH were also found to increase. These findings suggest that UVB/DSS treatment may induce the secretion of CRH and the activation of urocortin 2, causing the deterioration of ulcerative colitis. In order to prove this hypothesis, it will be necessary to investigate whether the expression or the activity of CRHR type 2 (a urocortin 2 receptor) increases in the region of inflammation in the colon. Moreover, it is necessary to examine the mechanism by which CRH and/or urocortin 2 expression are altered by UVB eye irradiation.

## CONCLUSION

The present study showed that UV eye irradiation altered the symptoms of DSS-induced ulcerative colitis. UVB eye irradiation caused the deterioration of symptoms, while UVA eye irradiation led to the improvement of the symptoms (Fig. 5). These responses suggest the possible involvement of CRH, ACTH, β-endorphin and urocortin 2, which are hormones of the pituitary gland system. Future studies are expected to elucidate a new mechanism of ulcerative colitis and may lead to the development of new therapeutic drugs.

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