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RAPID COMMUNICATION

Thalidomide effect in endothelial cell of acute radiation proctitis

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Abstract

AIM: To determine whether thalidomide prevents microvascular injury in acute radiation proctitis in white rats.

METHODS: Fourteen female Wistar rats were used: six in the radiation group, six in the thalidomide group, and two in normal controls. The radiation and thalidomide groups were irradiated at the pelvic area using a single 30 Gy exposure. The thalidomide (150 mg/kg) was injected into the peritoneum for 7 d from the day of irradiation. All animals were sacrificed and the rectums were removed on day 8 after irradiation. The microvessels of resected specimens were immunohistochemically stained with thrombomodulin (TM), von Willebrand Factor (vWF), and vascular endothelial growth factor (VEGF).

RESULTS: The microscopic scores did not differ significantly between the radiation and thalidomide groups, but both were higher than in the control group. Expression of TM was significantly lower in the endothelial cells (EC) of the radiation group than in the control and thalidomide groups ($P < 0.001$). The number of capillaries expressing vWF in the EC was higher in the radiation group (15.3 ± 6.8) than in the control group (3.7 ± 1.7), and the number of capillaries expressing vWF was attenuated by thalidomide (10.8 ± 3.5, $P < 0.001$). The intensity of VEGF expression in capillaries was greater in the radiation group than in the control group and was also attenuated by thalidomide ($P = 0.003$).

CONCLUSION: The mechanisms of acute radiation-induced proctitis in the rats are related to endothelial cell injury of microvessel, which may be attenuated with thalidomide.

Key words: Radiation proctitis; Von Willebrand factor; Thrombomodulin; Vascular endothelial growth factor; Thalidomide

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INTRODUCTION

Radiation therapy is performed widely in the suppression of the growth of malignant tumors, but it is occasionally accompanied by acute or late complications. Acute radiation injury to the intestine induces mucosal inflammation and damage to the endothelial cells (EC) of microvessels[1,2]. In radiation enteropathy, there may be a causal relationship between EC apoptosis and crypt cell apoptosis[3]. In addition to EC damage, increased chemotaxis and thrombogenesis of damaged vessels are the main mechanisms causing radiation enteropathy[1,3-6]. These events are related to complex molecular mechanisms involving many kinds of cytokines in the coagulation system, including thrombomodulin

(TM)[1,3,4]. In inflammatory bowel disease (IBD), microvascular inflammation also plays a crucial role in the pathogenesis[7].

In studies of IBD, thalidomide has been used to treat both animal models of colitis and human refractory Crohn's disease. Thalidomide suppresses the production of many kinds of cytokines including tumor necrosis factor-α (TNF-α) and it also suppresses angiogenesis[8-12]. Angiogenesis, or new vessel formation, is considered a novel target of therapy for IBD[7,13-15]. The factors promoting angiogenesis in IBD are tissue hypoxia and many kinds of cytokines secreted by infiltrating inflammatory cells[13]. We investigated the expression of von Willebrand factor (vWF), TM and vascular endothelial growth factor (VEGF) in acute radiation-induced proctitis and the response to thalidomide treatment.

MATERIALS AND METHODS

Experimental animals

Fourteen 10-wk-old female Wistar rats (body weight, 220-300 g) were used as the experiment subjects. White rats were purchased from Charles River Japan (Kanagawa, Japan), maintained in a standard steel net cage for 7 d (12-h light and dark cycle) at 24℃, and fed with standard animal feed and water. Three groups were assigned randomly: six animals were assigned to the radiation group, six animals to the thalidomide group, and two animals to the control group. Both the radiation group and thalidomide group were irradiated in the pelvic area with 30 Gy as a single dose. For the thalidomide group, thalidomide was dissolved in dimethyl sulfoxide at 10 mmol/L, and 150 mg/kg thalidomide was injected intraperitoneally for 7 d from the day of irradiation. On day 8 after irradiation, all animals were killed under anesthesia by injecting pentobarbital (40 mg/kg) and the rectum was removed from each animal.

Microscopic examination

The rectum was resected and examined macroscopically and then fixed in 10% formalin. The sample was dehydrated with ethanol, 7 μm-thick paraffin sections were cut, paraffin removed with xylene, and the sections stained with hematoxylin-eosin and examined. For microscopic examination, the hematoxylin-eosin-stained samples were evaluated by a pathologist who was unaware of the groups or treatment. Each sample was scored into one of five stages[16] as 0 = normal; 1 = slight radiation damage, mild inflammation or slight crypt change; 2 = mild damage, more significant inflammation, or crypt change; 3 = moderate damage, loss of epithelium, degree of inflammation variable; and 4 = severe damage (ulcers, necrosis).

Immunohistochemical staining

Tissue samples were immunohistochemically stained according to the manufacturer's protocols for TM using a polyclonal antibody (1:100; Santa Cruz, CA, USA), for vWF using a polyclonal antibody (1:600; Abcam PLC, Cambridge, UK), and for VEGF using a rabbit polyclonal IgG (1:300; Santa Cruz, CA). The intensity of VEGF and TM expression in endothelial cells of microvessels was measured separately at the mucosa and submucosa, and it was scored into one of four grades (none, weak, moderate, strong). Expression of vWF was measured quantitatively by observing five random areas of stained microvessels under the light microscope in both the mucosa and the submucosa, and is presented as the mean ± SD[17,18].

Statistical analysis

Parametric data were analyzed by Student's unpaired *t* test, and nonparametric data were analyzed by *F* test. *P* value less than 0.05 was considered significant.

RESULTS

Histological findings

All rats in the radiation and thalidomide groups showed grade 2-3 pathology, and this differed noticeably from the control group. The pathology (i.e. the change of crypt, the level of mucosal inflammation, and the infiltration of inflammatory cells) was more severe in the radiation and thalidomide groups than in the control group. The scores of the microscopic findings did not differ significantly between the radiation group (2.3 ± 0.5) and the thalidomide group (2.5 ± 0.4).

TM expression

The EC of normal controls showed only strong (3/4) and moderate (1/4) expression of TM. In the radiation group, there was weak (58%, 7/12) or no (42%, 5/12) TM expression with no samples of strong or moderate TM expression. The thalidomide-treated group showed moderate TM expression in 50% (6/12) of samples and strong expression in 50% (6/12) of samples. TM expression was significantly greater in the control and thalidomide groups than in the radiation group ($P < 0.001$, Table 1, Figure 1).

Intensity of vWF expression

The mean number of capillaries expressing vWF in both the mucosa and submucosa was 3.7 ± 1.7 in the control group, 15.3 ± 6.8 in the radiation group, and 10.8 ± 3.5 in the thalidomide group ($P < 0.001$). The radiation-induced capillary damage was significantly greater in the radiation group than in the control group, and the damage was reduced by thalidomide treatment (Figure 2).

Intensity of VEGF expression

VEGF expression was weak in all capillaries (4/4, 100%) of the control group. Most capillaries of the radiation group (11/12, 92%) had moderate expression except one severe case. Capillaries of the thalidomide group had weak (7/12, 58%) or moderate expression (5/12, 42%, $P = 0.003$; Table 1, Figure 3).

DISCUSSION

Acute radiation injury induced histological changes

Table 1 The intensity of TM and VEGF expression in EC in the mucosa and submucosa

	Control group				Radiation group				Thalidomide group				*P*
	0	+	++	+++	0	+	++	+++	0	+	++	+++	
TM	0	0	1	3	5	7	0	0	0	0	7	5	< 0.001
VEGF	0	4	0	0	0	0	11	1	0	7	5	0	0.003

0: None; +: Weak; ++: Moderate; +++: Strong expression.

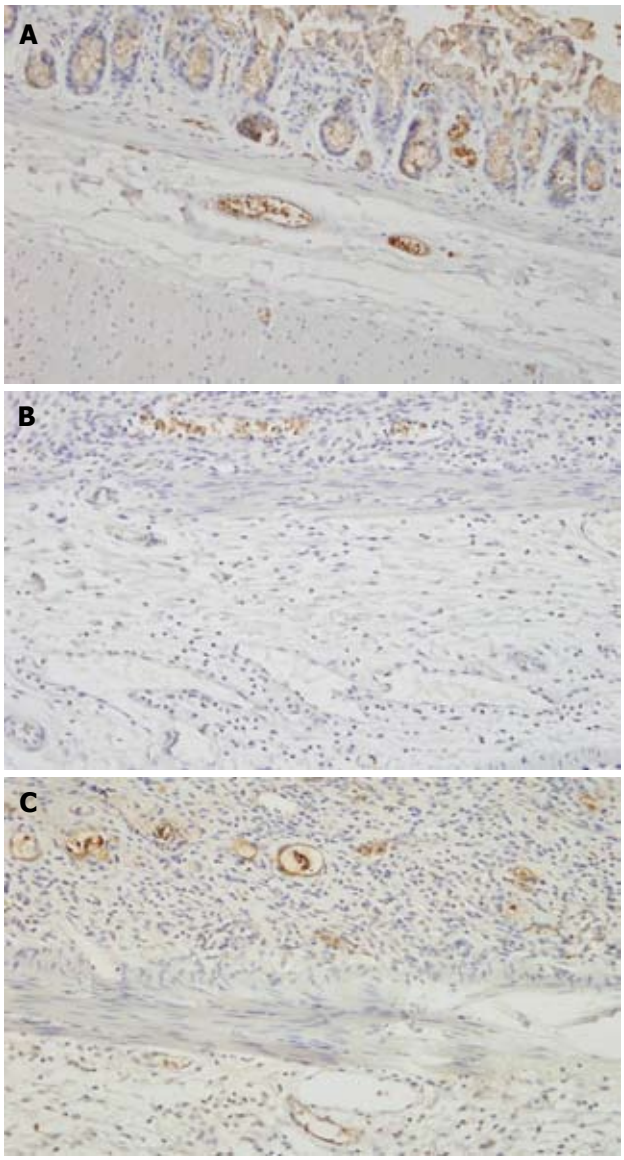

Figure 1 Immunohistochemical staining of TM (thrombomodulin) expression in the endothelial cells of the mucosa and submucosa. The intensity of TM expression in capillaries is significantly higher in the control group (**A**) and thalidomide group (**C**) than in the radiation group (**B**) (× 200).

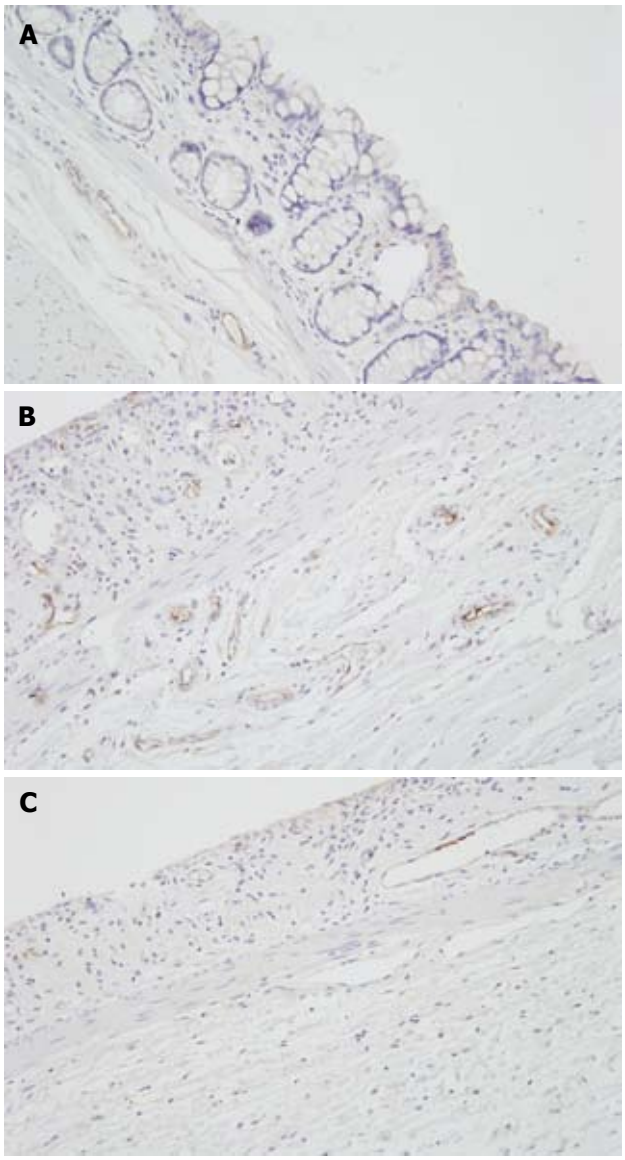

Figure 2 Immunohistochemical staining of vWF expression in the endothelial cells of the mucosa and submucosa. Both the total number of microvessels and the number of vWF expressing microvessels are markedly higher in the radiation group (**B**) than in the control group (**A**), which are attenuated in the thalidomide group (**C**) (× 200).

including edema, hyperemia, crypt abscess, ulcers, and necrosis of the intestine. It is thought that both acute and chronic radiation injury results from tissue ischemia secondary to thrombogenesis induced by thrombin formation in microvessels[1,6]. This loss of endothelial thromboresistance in radiation enteropathy may be related to the actions of many kinds of molecular markers such as tissue factor, vWF, platelet activating factor, TM, and prostacyclin 1[1,3-6].

In this study, the expression of TM in the EC was significantly lower in the radiation group than in the control group. These findings are consistent with previous studies suggesting that the mechanism of radiation-induced injury is related to endothelial thrombogenesis[1,6,19,20]. This reduced expression of TM in radiation group was restored by treatment with thalidomide. It is known that thalidomide treatment

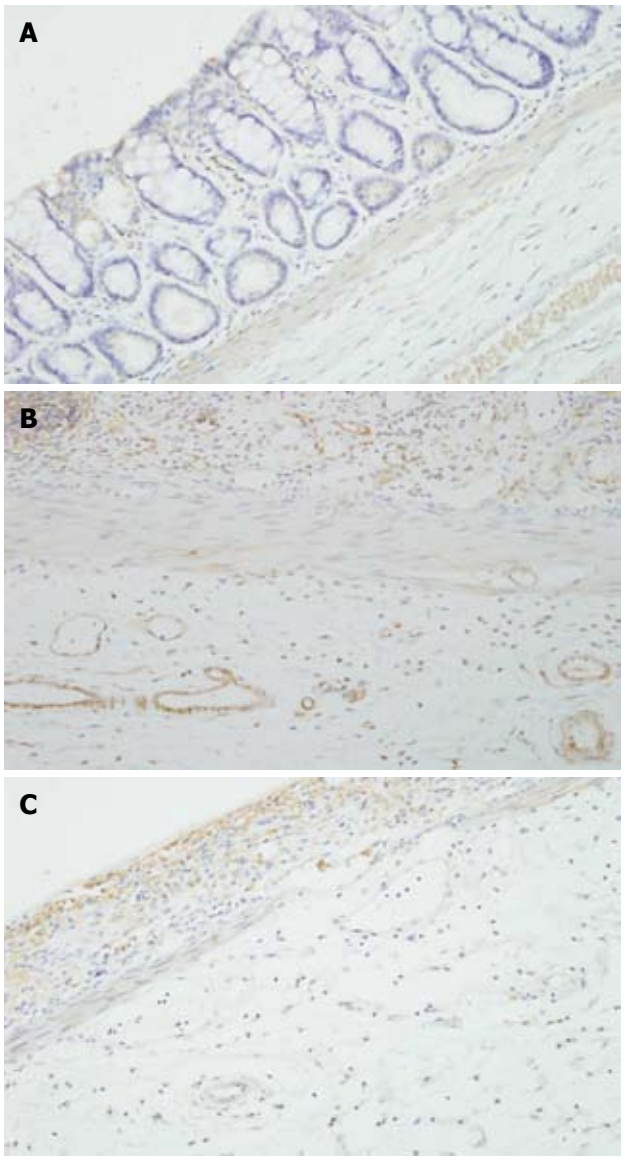

Figure 3 Immunohistochemical staining of VEGF expression in the endothelial cells of the mucosa and submucosa. The number of VEGF-positive microvessels and the intensity of VEGF expression were significantly higher in the radiation group (**B**) than in the control (**A**) and thalidomide (**C**) groups (× 200).

suppresses the increase in myeloperoxidase activity and production of TNF-α, interleukin-1β, and intercellular adhesion molecule-1 in an animal model of dinitrobenzene sulphonic acid-induced colitis and *in vitro* model *via* nuclear factor-κB signaling[10,12,21,22]. We found here that thalidomide treatment increased the TM expression in the EC of acute radiation proctitis. This finding might be due to the suppression of inflammatory cytokines including TNF-α by thalidomide. However, thalidomide may rarely cause thrombogenesis as a side effect when used in the treatment of multiple myeloma, although the underlying mechanism is not clear[23-26]. In our study, thalidomide seemed to contribute to thromboresistance rather than to thrombogenesis. If thalidomide is used to prevent radiation injury in the future, the clinician should consider that it can unintentionally cause aggravated enteropathy.

Because increased vWF expression in the EC is regarded as a marker of endothelial damage[27,28], we counted the number of vessels expressing vWF in the mucosa and submucosa of rats with radiation-induced proctitis. The number of vWF-expressing vessels was much higher in the radiation group than in the control group, but was reduced by thalidomide treatment. In the normal EC, the expression of vWF is low because high TM levels on EC surface degrade coagulation factor V and vWF[5]. Also in our study, some decrease of vWF expression in the EC of the thalidomide group might be explained by degradation of vWF by elevated TM.

We also found greater VEGF expression in the EC from the radiation group compared with the control group. It is thought that two factors induced by radiation injury contribute to elevated VEGF expression in EC. First, radiation-induced thrombus formation and ischemia of microvessels contribute to the increased production of angiogenic factors such as VEGF[29-32]. Secondly, inflammatory cytokines induce angiogenesis, and this may contribute to increased VEGF expression, as in other forms of IBD. This suggests that VEGF expression might be a candidate marker of radiation injury in radiation enteropathy, as in other IBD[13,15,33]. The effect of thalidomide on VEGF expression in the EC may be related to either direct inhibition of the EC, as shown in a study of human EC lines, or to inhibition by inflammatory cytokines of colitis, as in another colitis model[12,13]. In addition, it is thought that thalidomide might contribute to decreased VEGF expression by inhibiting thrombus formation by elevating TM production, as shown in our data. In other words, thalidomide may enhance the endothelial thromboresistance in radiation enteropathy, suggesting that thalidomide may help prevent radiation injury. We found no histological differences between the radiation and thalidomide groups, probably because of the near-complete denuding of all crypts in both the radiation and thalidomide groups. It is likely that the radiation dosage (30 Gy) was too high for thalidomide to salvage the radiation toxicity.

COMMENTS

Background

Tissue hypoxia or ischemia secondary to the microvessel damage and coagulopathy has been considered as a main mechanism of radiation injury in various organs including intestine. Thalidomide as anti-angiogenic or anti-TNF-α agent, has been used for the treatment of malignant disease and inflammatory bowel disease (IBD). Several studies about thalidomide therapy support that it might be useful to prevent radiation-induced enteropathy.

Research frontiers

Recently, a few studies have been reported about both tissue hypoxia and microvessel injury in radiation injury of the intestine with related molecular markers such as vascular endothelial growth factor (VEGF).

Innovations and breakthroughs

To evaluate the effect of thalidomide for prevention of radiation-induced proctitis, the markers related to radiation-induced vascular damage were investigated. Our results show that thalidomide has beneficial effects in these markers in spite of no salvation of microscopic damage.

Applications

By applying the markers of microvascular injury [von Willebrand Factor (vWF),

VEGF and thrombomodulin (TM)] in radiation-induced proctitis, we provide scientific basis for the thalidomide therapy in the prevention of acute radiation-induced proctitis. In the future, it could be useful as a preventive agent for the prevention of radiation-induced proctitis although thalidomide has some vascular side effects.

Peer review

The manuscript describes that irradiation to the rectum induces vWF and VEGF in vascular endothelial cells and inhibits TM expression as a marker of thromboresistance. Thalidomide treatment attenuates the effects of the radiation injury in endothelial cells.

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