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Laboratory Investigation

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Expression of Cytokines in Radiation Injured Brain at Acute Phase

Objective : Radiation therapy is an important treatment for brain tumor. However, serious complications such as radiation necrosis can occur and it may be secondary to the expression of acute phase genes, like cytokines. In particular, inflammatory cytokines (IL-1β, TNF-α) and other immunomodulatory cytokines (TNF-α, TGF-β1) might be changed after irradiation (high single dose irradiation). Although it has been reported that IL-1 level is remarkably elevated within 8 week after the irradiation to the rat brain, the change of cytokines levels at acute phase (within 24 hours) has not been reported. In the present study, we examined TNF-α, TGF-β1, and IL-1β levels in acute phase to clarify the early effect of cytokines on the radiation-induced brain damage.

Methods : Fifty Sprague-Dawley rats were used and these were divided into irradiation group and control group. After a burr-hole trephination on the right parietal area using a drill, a single 10 Gy was irradiated at the trephined site. Their forebrains were extirpated at 30 min, 2 hr, 8 hr, 12 hr and 24 hr, respectively and examined for the expression of TNF-α, TGF-β1 and IL-1β.

Results : The expression of TNF-α and TGF-β1 were decreased until 12 hr after irradiation but elevated thereafter. The expression of IL-1 was peak at 8 hr and then decreased until 12 hr but elevated after this time window. The present study indicated that expression of cytokines (TNF-α, TGF-β1 and IL-1β) were increased at 24 hr after the irradiation to the rat brain. IL-1β level, on the other hand, reached peak at 8 hr after radiation injury.

Conclusion : These findings indicate that IL-1, among various cytokines, may have a more important role in the inflammatory reaction by radiation injury at acute phase and provide some clues for better understanding of the pathogenesis of radiation injury.

KEY WORDS : Radiation · Brain injury · TNF-α · TGF-β1 · IL-1β.

INTRODUCTION

Patients who undergo radiation therapy for brain diseases often suffer from side effects such as acute edema, demyelination and prolonged radionecrosis[7,15,25]. However, exact causes and mechanisms of cerebral histological and molecular changes after radiation treatment have not been elucidated although it has been reported that acute phase genes express, within several hours after radiation, intercellular adhesion molecule-1 that may associate with these changes[10].

Generally, cytokines regulate proliferation and differentiation of normal and malignant glial cells. These cytokines play an important role in normal and pathological cerebral reactions including inflammatory diseases and multiple sclerosis in central nerve system (CNS)[6,9,21,26,29]. Radiation, also, is a strong stimulant for inflammatory reaction. Accordingly, after radiation, expressions of several cytokines are reported to be increased[4,11]. It was also reported that cytokines produce by glial cell and microglia play an important roles in inflammatory reaction at the latent period before prolonged radiation injuries in irradiated brain[5]. Therefore, if mechanism of cytokines in brain tissue after radiotherapy can be revealed, it may provide an important clue to prevent a complication through regulating expression of cytokines. Hence, the aim of this study was to elucidate the expression of various cytokines (e.g., IL-1β, TNF-α and TGF-β1) at acute phase in normal rat brain after irradiation.

MATERIALS AND METHODS

Experimental animals

Fifty female Sprague-Dawley rats weighing 200-300 g were used. The rats were given water and food ad libitum until the experiment was conducted.

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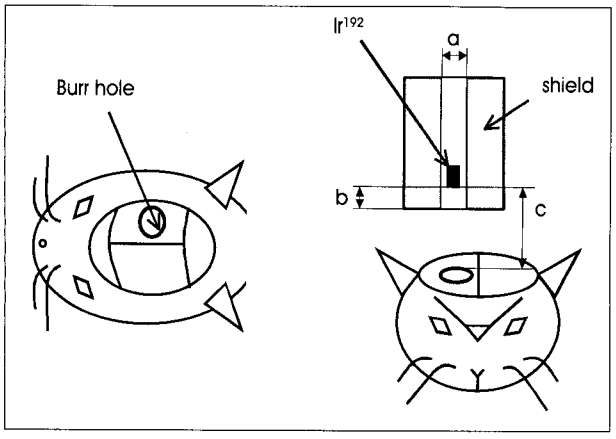

Fig. 1. Schematic Drawing of Irradiation. a : diameter of radiation tube; 0.6 cm, b : distance from the radiation source to the distal cone; 0.5 cm and c : distance from source to the brain surface; 2 cm.

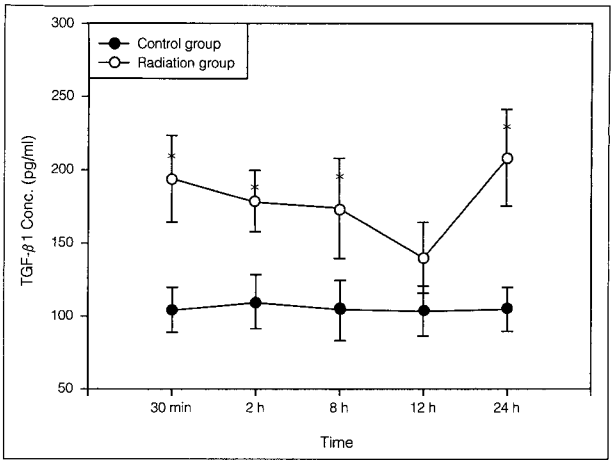

Fig. 3. Concentration of transforming growth factor-β1 (TGF-β1) according radiation injured brains. All values are expressed as means ± SD ($p < 0.05$).

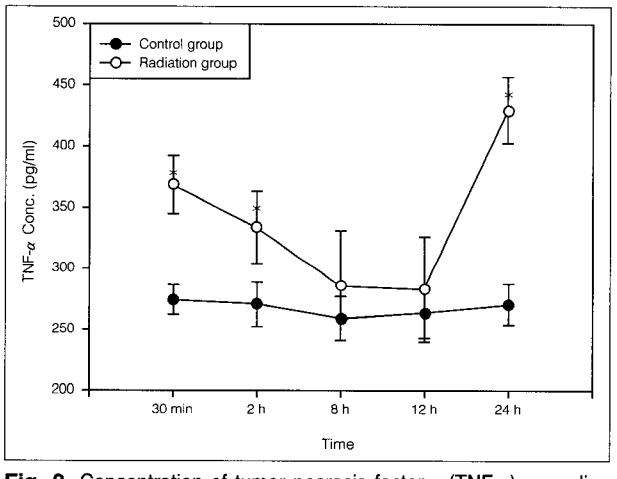

Fig. 2. Concentration of tumor necrosis factor-α (TNF-α) according radiation injured brains. All values are expressed as means ± SD ($p < 0.05$).

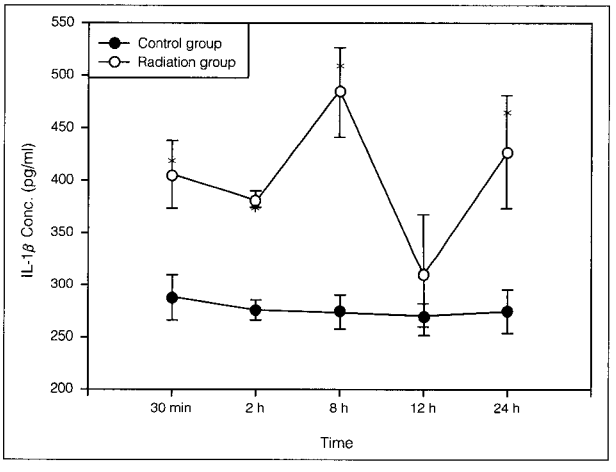

Fig. 4. Concentration of interleukin-1β (IL-1β) according radiation injured brains. All values are expressed as means ± SD ($p < 0.05$).

Single high-dose irradiation and forebrain extirpation

One percent ketamine (50 mg/kg), known to have no radioprotective effect, was intraperitoneally injected and then the heads were fixed to a head holder (SN-8N Semichronic Head holder, Narishige Co., Tokyo, Japan) in a ventral decubitus position. A longitudinal incision was made on the scalp along the median line. Under the operative microscopy, an 8-mm diameter hole was drilled on the right parietal area to expose the dura mater (Fig. 1). Because it was technically impossible to conduct intraoperative irradiation on a small rat brain using electron beams, iridium-192 (GammaMed 12i, MDS Nordion, Canada), which has the same characteristics as the electron beam and is commonly used for high-dose rate brachytherapy, was used as a source of radiation. A shield was made of Lipowitz metal (Cerroben®, MED-TEC, Orange, IA, USA), and then a 0.6-cm hole was made in the middle to ensure that the iridium source was located 0.5 cm above the hole. The source was located at sufficiently distant site so that the impact of diffused beams on the irradiation site could be minimized. About 1-cm diameter of the rat brain surface was exposed to radiation, and 10 Gy was irradiated on the brain surface 2 cm away from the source by a single irradiation (Fig. 1). The 10 Gy dose was selected because it was known as the lowest dose to have a radiation effect during intraoperative irradiation. After irradiation, the scalp was sutured in an aseptic condition with absorbable thread.

Twenty-five rats were divided into 5 groups, and their forebrains were extirpated at 30 min, 2 hour, 8 hour, 12 hour, and 24 hour after irradiation. In the control group, 25 non-irradiated rats also underwent forebrain extirpation at the same time intervals as the irradiation group. The extirpated brains were washed by phosphate buffered saline (PBS) and were stored at -70°C until used.

Protein extraction

The brain tissues from each group were mixed with pre -chilled lysis solution [0.1 M sodium phosphate, pH 7.2, 1 mM phenylmethylsulfonylfluoride, 10 mM EDTA, 10 mM EGTA, 10 mM NaF, 1% (w/v) sodium deoxycholate, 1% (v/v) Triton X-100, 0.1% (w/v) SDS] containing 200 kallikrein inhibitor units of aprotinin/ml). The mixture was pipetted and incubated for 30 min on ice, and centrifuged at 10,000 x g for 15 min at 4℃. The supernatant was stored at -70℃ until used.

Concentrations of IL-1β, TNF-α and TGF-β1

IL-1β was determined by IL-1β DuoSet ELISA Development kit (R&D Systems, Inc., USA). 96 well plates were coated with IL-1β coat mAb and the captured IL-1β was bound by a second specific polyclonal antibody. The amount of specifically bound pAb was detected using a species-specific antibody conjugated to horseradish peroxidase. The color development was stopped and the intensity of the colors was measured at 450 nm. Measurement of TNF-α and TGF-β1 in the protein solution was analyzed by the same method, except for specific antibodies.

Statistical analysis

The expressions of IL-1β, TNF-α and TGF-β1 from each group were statistically analyzed using the Duncan method of the one-way ANOVA procedure of SPSS (2004). For each group, the mean and SD for IL-1β, TNF-α and TGFβ1 were calculated. Significance was determined at the $p<0.05$ level.

RESULTS

Expression pattern of TNF-α in irradiated brain

To evaluate the expression of TNF-α after irradiation in normal rat brain, the expression of TNF-α was compared with that of different periods by ELISA assay (Fig. 2). TNF-α expression of the irradiated brain at early stage (30 min) was higher than at 8 hr and 12 hr, showing a peak at 24 hr (431.58$\pm$27.09 pg/ml, $p<0.05$). But, in control group, differences in the brain TNF-α level were not detected. And, the TNF-α expression of radiation group was higher than those of the control group except for 8 hr and 12 hr ($p<0.05$).

Expression pattern of TGF-β1 in irradiated brain

The least square means of the TGF-β1 concentration of irradiated brain are shown in Fig. 3. TGF-β1 level of irradiated brain decreased slowly until 12 hr (194.93$\pm$29.54 to 141.11$\pm$24.16 pg/ml), then showing a peak at 24 hr (209.09$\pm$32.81 pg/ml). However, differences in the normal brain TGF-β1 concentration also were not detected. TGF-β1 expression of radiation group was higher than those of the control group except for 12 hr ($p<0.05$).

Expression pattern of IL-1β in irradiated brain

Fig. 4 shows the changes with time of IL-1β concentration in irradiated brain. IL-1β concentration of irradiated brain showed peak at 8hr (485.99$\pm$43.12 pg/ml) and then decreased. But, it was increased again after 12 hr (310.52 $\pm$52.98 to 428.56$\pm$53.63 pg/ml). The differences in the normal brain IL-1β concentration also were not detected. IL-1β expression of radiation group was higher than those of the control group except for 12 hr ($p<0.05$).

DISCUSSION

Irradiated brain injuries cause many symptoms including temporary functional disorder and organic brain change, but pathophysiological mechanisms have not been understood clearly. The pathological radionecrosis was classified into three stages. First, acute stage damage was taken ill within several days after radiatiation and this was accompanied with temporary cerebral edema. Secondly, subacute stage damage was observed symptoms within several weeks and this stage damage was accompanied with demyelination of white matter. Finally, late stage damage or delayed brain injury was observed symptoms within several months to years[12].

It has assumed that the disorder of cerebral microcirculation raise the radiation necrosis and certain cytokines and immunomodulatory factors participate in increasing vascular permeability, astrocytoma, and gliomatous proliferation. However, these mechanisms have not been reported.

Calvo et al.[3] reported that the incidence rates of the white matter necrosis after irradiation depended on the radiation exposure dose and the process of time after radiation[3]. Although, irradiated normal brain did not extensively changed within 24 hr, our previous study has shown that over the subacute stage after irradiation of 10 Gy on normal rat cerebral tissue, fibrous gliosis, appearance of inflammatory cells, proliferation of vascular endothelial cells, and necrosis gradually increased over time[11]. Recently, Kureshi et al.[12] reported that TNF-α, TGF-β and IL-6 levels significantly increased in brain tissue after radiotheraphy.

TNF-α is a 17 kDa polypeptide, mainly is produced by a macrophage and a monocyte. Generally, it is an immune-mediator to protect the host from infectious agents and cancer cells. Particularly, TNF-α induces the cancer cell death by cellular apoptosis. It also induces cytokines expression

including IL-1 and IL-6 in the vascular endothelial cell and cancer cell. Neta et al.[20] reported that TNF-α injected rats before radiation showed higher survival rates than those without protection effect of hematopoetic system. This study has also shown that TNF-α expression in the irradiated rat brain was higher than those of the control group except for 8 hr and 12 hr ($p<0.05$). These results suggest that TNF-α play an important role in the irradiated brain by regulating physiological radiation resistance and restoration.

TGF-β is a multifunctional polypeptide and is mainly produced by an astrocyte and a macrophage. It has five subtypes. Of these, TGF-β1 is known to bifunctionally regulate the generation and progress of cancer by inhibition or promotion of proliferation. This is recently evidenced by Seong et al.[27] that TGF-β expression dose dependently increase in rat liver after radiation.

IL-1β is a 17 kDa proinflammatory cytokine, is mainly produced by a microgliacyte and macrophage and induces the proliferation of macrophage and astrocyte[1,24]. Also, IL-1β influences the production of gliocyte[13,14], and the effect of cytotoxicity about oligodendrocyte[2,19,28]. Moreover, it induces expression of IGF-I (8 and 18) that are known to regulate the regeneration of nerve tissue[17,30].

In this study, the expression of cytokines (TNF-α, TGF-β1 and IL-1β) was compared with that of different periods by ELISA assay to evaluate the expression of cytokines of acute stage (30 min-24 hr) after irradiation in normal rat brain. The cytokine expression of radiation group was higher than those of the control group in almost times. These results suggest that such cytokines may play an important role in the damaging or recovery of irradiated brain. Kureshi et al.[12] reported that the increment of cytokines expression indicated that these cytokines conspired to influence each other according to situations of surrounding cells and tissues rather than acted by each function. These interactions may lead multifunctional effects including proliferation of astrocyte and vascular endothelial cell and destruction of blood-brain barrier.

Taken all study results together into consideration, various cytokines (TNF-α, TGF-β1 and IL-1β) contributed to the irradiated injury, namely glial proliferation, damage of blood vessel, and tissue necrosis after 24 hr. In particular, IL-1β level showed peak at 8 hr after radiation injury. These findings indicate that IL-1 may have a more important role in the inflammatory reaction by radiation injury at acute phase and will provide some clues for understanding of the pathogenesis of radiation injury.

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