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The Effect of Total Radiation Dose on Normal Spinal Cord of Hybrid Mice

—Early Pathological Changes—

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Radiation myelitis is a rather rare, but irreversible fatal complication. Etiology, pathologic change, clinical symptoms and the method of diagnosis have been studied with animal experiments and human necropsies. In spite of massive studies, pathogenesis of post-irradiation myelitis and the level of tolerance dose still remain controversial.

Thoracolumbar spine of 110 hybrid mice were irradiated with orthovoltage x-ray machine.

Mild capillary congestion and axonal swelling were observed in 1,000 rad irradiated specimens. Focal necrosis in 3,500 rad specimens, fragmentation of neural tissue in 4,000 rad specimens were also observed.

These results suggest that 5,000 rad is not a completely safe tolerable dose which have been accepted and we cannot exclude direct radiation damage to nerve tissue as the causative pathology of radiation myelitis in addition to blood vessel damage.

Key Words: Acute radiation myelitis, Radiation pathology.

INTRODUCTION

Radiation myelitis is a rather rare complication following radiation therapy, but it may result in severe irreversible, sometimes fatal condition.[1~4] Many in vivo and in vitro experiments were performed in attempts to discover the nature of these changes, and also many human cases of radiation myelitis have been reported even in present time.[5] Particularly, in head and neck cancer treatment, the irradiated dose at the site of juctional fields is considerably higher because of the beam divergence from multiple fields. These beam divergence is aggaravated by flexion or extension of the patient's neck so that careful treatment planning is very important.[6]

Human CNS system has been known "relatively radioresistant"[7], and 5,000 rad in sweeks have been accepted as a tolerable dose[6~8]. But recently, radiationmyelitiswasreportedatthelevelofbelow 3,500 rad,[9] and some authors insist "3,300 rad in 42 days"[6] or "3,500 rad in 17 fraction"[8] as the safe dose, these suggest that 5,000 rad in 25 fraction may not be safe completely.

In spite of those importance, little information on threshold dose, tolerance dose, latent period,[5,6,8,10] and exact pathological mechanism are still controversial. Also, most clinical reports of transverse myelitis depend on necropsy or autopsy findings and most animal experiments were drawn from small fraction or single fraction. Therefore, there will be some differences between clinical and experimental situation.

The purposes of this article are:

1. analysis of the histopathological findings at the therapeutic dose level with a manner similar to radiation therapy of human,
2. to estimate the minimum dose level which express irreversible damage,
3. to predict the dose-reponse relationship which early injury could be used as a scale for later development of irreversible, chronic spinal injury.

This study is the analysis of only "early acute responses", so study of chronic, delayed finding should be followed.

MATERIAL AND METHOD

Used animals were 110 hybrid white mice (55 male, 55 female) weighing 25±1 gm of male, 23± 2 gm of female, 30±3 day old, respectively. These selection were based on previous our experiments which were already published.[11,12] 10 groups animals except 1 control group were irradiated with various doses from 1,000 rad to 5,000 rad with 500 rad increment. Irradiation was performed under light ether anesthesia with 250 kVp, 25 mA (Coronado, Westinghous, England) orthovoltage x-ray machine. Conventional fraction (200×5/wk), 2×3 cm field were used and details of the procedures are given elsewhere.[12]

After adequate irradiation, animals were sacrified immediately and mid lumbar spinal cord was excised for histological specimens. All specimens were examined by 2 pathologist to minimize subjectivity of findings.

RESULT

No significant gross abnormal movement or sign of paralysis were observed throughout experiments. Histopathological findings at various irradiated dose level were summarized in Table 1, and the details are as follows.

In 500 rad group, no significant difference from control group was observed in male and female specimens.

In 1,000 rad group, focal infiltration of inflammatory cells between white and gray matter and slight axonal edema were observed in male and female specimens.

In 1,500 rad group, increased infiltration of inflammatory cells than 1,000 rad group were observed. Slight proliferation and congestion of the capillary were also observed which prominant in female specimens. Focal cytoplasmic vacuolization was observed in some specimens (Fig. 1).

In 2,000 rad group, capillary proliferation and congestion were evident. Slight sponge degeneration in white matter and increased cytoplasmic vacuolization were observed (Fig. 2).

In 2,500 rad irradiated group, moderate capillary congestion and spongiosis of white matter were observed in male and female specimen. Mild focal axonal swelling was also observed.

In 3,000 rad irradiated group, marked cytoplasmic vacuolization and sponge degeneration as well as capillary congestion and dilatation were observed. Slight focal thickening of pia and arachnoid was also observed in some specimens (Fig. 3).

In 3,500 rad irradiated group, severe capillary congestion and increased sponge degeneration were observed. Focal sponge necrosis was seen in one female specimen.

In 4,000 rad irradiated group, fragmentation and focal loss of neuron were observed. Focal sponge necrosis was evident in male and female specimens. Thickening of pia and arachnoid was also observed (Fig. 4).

In 4,500 rad irradiated group, increased focal necrosis and fragmentation were of neuron observed.

In 5,000 rad irradiated group, focal loss of neuron, focal necrosis and spongiotic change were increased in male and female specimens (Fig. 5).

Table 1. Histopathological Changes of Normal Spinal Cord of Hybrid Mice after Radiation

Dose of radiation (rad) / Histopathological changes	500	1,000	1,500	2,000	2,500	3,000	3,500	4,000	4,500	5,000
Focal infiltration of inflammatory cells		±	+	++	++	++	+++	+++	+++	+++
Capillary proliferation and congestion		±	±	+	++	++	+++	+++	+++	+++
Focal axonal swelling		±	±	+	+	++	++	++	+++	+++
White matter sponge degeneration				±	+	+	++	++	++	+++
Pia and arachnoid thickening						±	±	+	+	++
Focal necrosis							±	+	++	++
Neural tissue fragmentation								±	+	+
Focal loss of neuron								±	+	++
Focal reactive gliosis									±	+

±: Tracely positive, +: Mild, ++: Moderate, +++: Severe

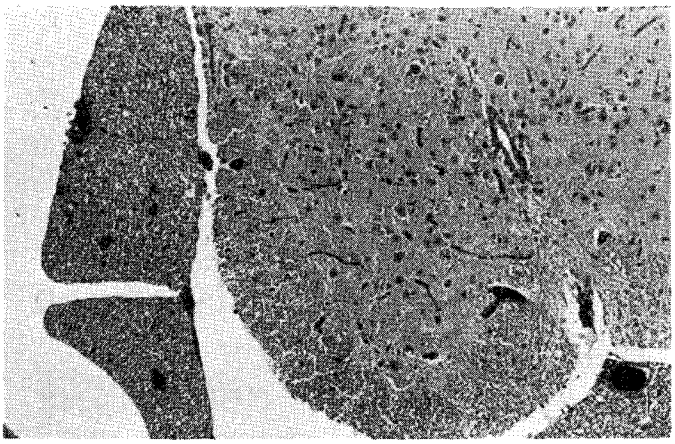

Fig. 1. Slight congestion and proliferation of the capillaries in 1,500rad irradiated group (H&E, X40).

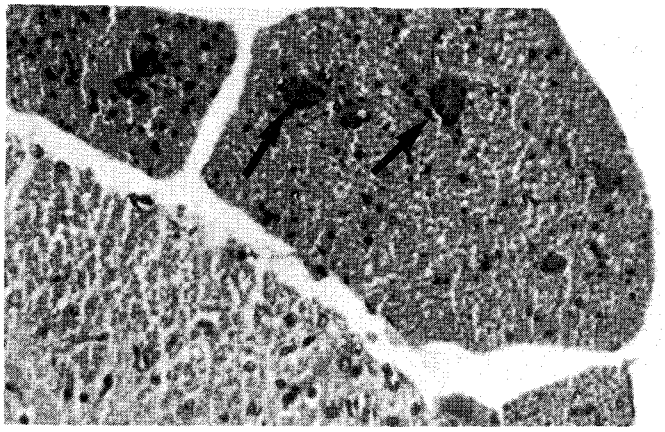

Fig. 2. Marked increased capillary congestion in 2,000rad irradiated group (H&E, X100).

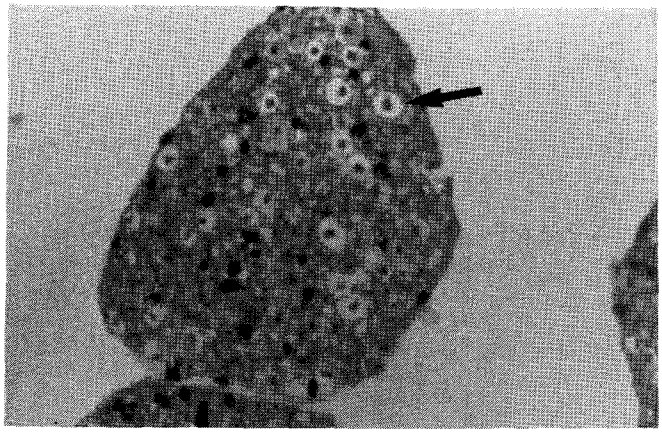

Fig. 3. Marked increased cytoplasmic vacuolization in 3,000rad irradiated group (H&E, X200).

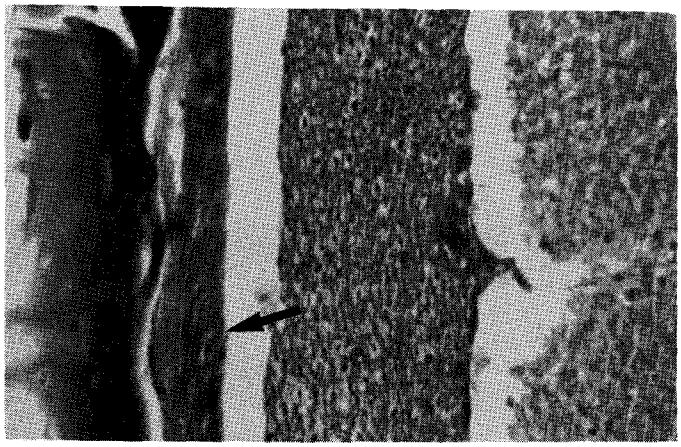

Fig. 4. Focal thickening of arachnoid in 4,000rad irradiated group (H&E, X100).

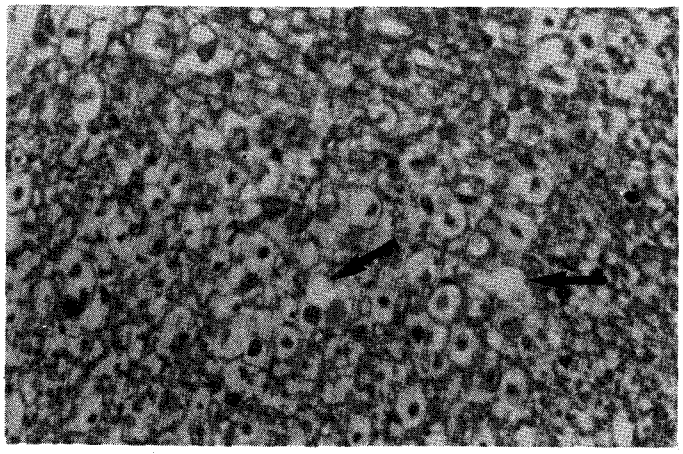

Fig. 5. Focal loss of neuron (arrows) and spongiotic changes in 5,000 rad irradiated group (H&E, X400).

DISCUSSION

It has been known that therapeutic dose level of radiation can cause serious damage to nervous tissue throughout many experimental and clinical reports. Exact diagnosis is very difficult because radiation injury to nervous tissue produce various symptoms which is very similar to metastasis, and the latent period is very irregular, sometimes very long (1 month[1]-27 years[13]).

Pallis et al[14] proposed 3 criteria for a diagnosis of radiation myelopathy; 1) spinal cord was included in irradiated area, 2) main neurological lesion was within the segments of cord exposed to the radiation, 3) myelopathy or necropsy excluded cord compression from metastasis as the cause of neurological disorder.

Reported symptoms were tingling sensation and numbness of extrimities,[7,14~18] Lhermitte's sign,[6,8,14~17] loss of position sense, loss of temperature and pain sense, Brown-Sequard syndrome,[7,15] paraplegia and sphincter disturbance.[7,9,15,18]

Reagan et al[16] classified 4 categories for these symptoms: in type I (early transient myelopathy of Lhermitte), symptoms apppear in a few months and disappear in a few months which are purely subjective and hard to evaluate in animal experiments. In type II (sign of lower motor neuron of

extrimities), sensory loss and flaccid paralysis of extrimities are probably due to selective damage to anterior horn cells. In type III (acute paraplegia), acutely developing paraplegia or quadriplegia, presumably the result of infarction of spinal cord, secondary to vascular changes, occurs in a few hours, which was reported only in animal experiments. In type IV (chronic progressive myelopathy), most patients have multisystemic disease of the cord, such as muscle weakness, sensory impairment, bowel and bladder dysfunction, which progresses to death due to associated bronchopneumonia or urinary tract infection.

Although authors inspected neurological motor function of mice once in a week, no significant abnomality was observed. The reasons of these results were thought to be relatively low radiation dose (maximum was below 5,000 rad) and no enough latent time because of immediate sacrifice of animals after irradiation.

Various influencing factors have been proposed for the radiation myelitis, but Fletcher and Wilson[17] described followings as important factors: 1) total radiation dose, 2) rate of application, 3) extent to which the cord is shielded, 4) individual susceptibility and variabilitiy, 5) amount of tissue irradiated, 6) vascular supply to the area irradiated, 7) source of radiation. They claimed that the rate of application was most important among those factors. But Ang et al,[5] Kim and Fayos,[6] Fitzerald et al,[8], van der Kogel,[9], and Hubbard and Hopewell[10] insisted that the most important factors were total irradiation dose and fractionation. These support our hypothesis that different fractionation, even if NSD (nominal standard dose) is constant may result in different responses, so that experiment should use same fractionation as clinical treatment which we want to compare with.

However, 5,000 rad/25 fractions in 5 weeks has been usually accepted as a tolerable dose,[8] but Coy et al[15] and Boden[19] insist that tolerable dose should be reduced to 3,500 rad in 17 days (Boden), 3,300 rad in 42 days with large field irradiation (Coy).

Authors observed sponge degeneration and focal sponge necrosis in 3,500 rad irradiated group which is similar to Boden's result.

The pathogenesis of radiation myelitis remains still controversial. Maier et al,[7] Fitzerald et al[8] and Hubbard and Hopewell[10] emphasized that nerve damage is a product of direct radiation damage to glial cells, particularly oligodendroglial cells. But Kim and Fayos,[6] Coy et al,[15] Burns et al,[20], Backlund et al[21] and Godwin et al[22] stressed that radiation myelitis was secondary to vascular endothelial lesions in capillaries and smaller arteries. Fogelholm et al[23] have shown that although cellular degenerative changes due to ischemia do occur, nerve cell damage can also be demonstrated when the blood vessels are apparently undamaged. Fletcher and Wilson insisted that sufficient high dose could cause direct necrosis of nerve tissue but lower dose might produce slowly developing blood vessel changes, leading to later focal necrosis. Jellinger and Strum[18] insist that "idiosyncrasy" may be involved because radiation myelitis appeared even at below the necrosis level in his 6 case reports.

Our experiment showed that capillary congestion and axonal edema appeared in 1,000 rad group, infiltration of inflammatory cells which increased progressively by total dose increment. These suggested that direct damage to nerve tissue as well as vascular damage cannot be excluded as the pathogenesis of radiation myelitis.

CONCLUSION

110 hybrid mice (55 male, 55 female) were irradiated with large abdominal field, conventional fraction by 250 KV orthovoltage x-ray machine. The histopathological changes at the various total dose were analyzed.

1. Slight capillary congestion, focal infiltration of inflammatory cells and axonal edema appeared in 1,000 rad irradiated group.
2. Sponge degeneration of white matter appeared in 2,000 rad irradiated group.
3. Focal thickening of pia and arachnoid appeared in 3,000 rad irradiated group.
4. Focal necrosis was observed in 3,500 rad irradiated group.
5. Fragmentation and focal loss of neuron appeared in 4,000 rad irradiated group.
6. All above findings were aggravated progressively by increment of total dose.

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＝국문초록＝

총 방사선 조사량이 잡종 백색 마우스의 정상 척수에 미치는 영향

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최 원 희

방사선에 의한 척수의 손상은 매우 드물기는 하나 치명적인 불가역성의 손상을 일으킬 수 있는, 치료방사선 분야에서 가장 심각한 합병증의 하나이다.

동물실험 및 임상보고를 통하여 많은 연구가 있었음에도 실제의 임상에서와 같은 조사방법을 사용한 계통적인 보고는 거의 없는 실정이다.

이에 저자는 전 조사량에 대한 초기의 급성 변화를 병리조직학적으로 관찰 분석하여 불가역성의 척수손상이 올 수 있는 최저선량을 추정하기 위하여 250 KV의 X선 치료기를 사용하여 총 110마리의 마우스를 2×3 cm의 조사야로 고식적 분할조사를 실시하였으며 그 결과는 다음과 같다.

경한 모세혈관의 울혈, 염증성 세포의 부분적 침윤과 함께 축색돌기의 부종이 1,000 rad 군에서 관찰되기 시작하였다.

백질의 스폰지 변성은 2,000 rad 군에서 보이기 시작하였다.

연막과 지주막의 부분적 비후는 3,000 rad 군에서관찰되었다.

국소 괴사는 3,500 rad 군에서 관찰되기 시작하였고 신경조직의 단열과 신경원의 국소 손실은 4,000 rad 군에서 관찰되기 시작하였다.

이제까지 용인되어온 척수의 내성선량인 5,000 rad/5 wk.는 완전히 안전하지는 못하다고 생각되나 이는 만성 변화에 대한 추적실험을 통하여 확인되어야 할 것이다.