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The Effect of Irradiation and Cis-diamminedichloroplatinum(II) in the Rat Brain : Analysis of Histopathology at 3 and 6 Months after Treatment

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Purpose : To evalute the late effect(3 and 6 months) of cis-diamminedichloroplatinum(II)(cisplatin) on the radiation brain damage when the cisplatin was intraperitoneally infused immediately after whole brain irradiation in the rats.

Materials and Methods : The histolopathological findings of the brain were examined in rat brains at 3 and 6 months after the treatment. The rats were irradiated(20 or 22.5 Gy, RT) or cisplatin was injected intraperitoneally(2, 4, or 8mg/kg, CT) and in combined treatment group, cisplatin(2mg/kg) was injected immediately after irradiation(20 or 22.5 Gy). Histopathological examination was done mostly in irradiation or cisplatin alone groups, because the rats in combined group died during experimental period except 2 rats.

Results : The rats treated with cisplatin showed marked epithelial vacuolation with perivascular edema and vascular dilatation in choroid plexus at 3 months as well as multifocal necrosis involving fimbria and cerebellar hemispheres at 3 and 6 months. The changes were more prominent in rats with 2mg/kg injection compared to rats with 8mg/kg injection.The rats with RT and combined CT and RT showed characteristic delayed irradiation effects such as focal coagulation necrosis and vascular changes, which were more marked than previous reports. Prominent perivascular and leptomeningeal astrocytic proliferation was well documented by anti-GFAP antibody. Cisplatin treatment did not enhance the effect of radiation-induced changes of blood vessels and astrocytic proliferation.

Consluion : The focal necrosis was the most consistently noted finding in this study, it suggested the possibility to use this as an evaluation factor for combined effects of RT and cisplatin.

Key Words : Radiation, Cisplatinum, Rat brain

INTRODUCTION

Cis-diamminedichloroplatinum(cisplatin) is a heavy metal compound with a well established antitumoral effect in various tumors including brain tumors.[1-3] The effect of cisplatin as a potential

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radiosensitizer in brain tumor is controversial. In a rat brain tumor study, Douple et al. demonstrated an improvement over radiation therapy alone by the addition of systemic cisplatin.[4] Clinically, concomitant cisplatin has been effectively added to standard fractionation radiation therapy for the treatment of brain metastasis. However, an EORTC Brain Tumor Group Study concluded that systemic cisplatin did not enhance the effect of radiation therapy in malignant glioma. Combined cisplatin and cranial irradiation may lead to increased risk of ototoxicity, cranial nerve palsies, and coma.[5]

The changes occuring in normal brain parenchyme by treatment is important to know, since the effects of therapeutic irradiation and chemotherapy are inevitably limited by the tolerance of the surrounding normal tissue. Previous studies on effect of cisplatin alone or combination of cisplatin and radiation in the rabbit's brain[6, 7] did not show any clinical symptoms or histopathological changes. In their studies, histopathological examination was done at various times up to 2 weeks after intracarotid(IC) injection in cisplatin alone group and up to 8 weeks after irradiation in combination group. On the contrary, focal brain necrosis asssociated with increased permeability following IC administration of cisplatin was observed in other studies[8, 9] Intraperitoneal(IP) administration of more than LD50(12mg/kg) of cisplatin did not show histological changes in rat brain during 1-28 days in other study.[10]

The purpose of this study was to establish the morphologic effect of cisplatin alone or combined cisplatin and irradiation to the normal rat's brain at 3 and 6 months. In addition, histopathological findings of irradiation alone group were compared with previous studies. Astrocytic glial reaction was evaluated by glial fibrillary acidic protein(GFAP) immunoreaction and electron microscopic examination was performed at 6 months after treatment.

MATERIALS AND METHODS

1. Animals and experimental design

Sprague-Dawley rats of both sexes weighing 200-250gm were used for the present study. Each rat was allowed free access to food and water before and after the irradiation and chemotherapy. Sixty seven rats were devided into 3 groups: irradiation(RT) alone, chemotherapy(CT) alone, and combined irradiation and chemotherapy(Table 1).

Before the irradiation, the rats were lightly anesthetized with ketamine(50mg/ml) intraperitoneal injection(100mg/kg). Whole brain was irradiated 20 or 22.5 Gy by 6MV linear accelerator(NEC 1000×, Japan) through anterior 1 portal with shielding of both eyes.

In chemotherapy alone group, cisplatin(2, 4, and 8mg/kg, Cisplan, Dong-A Pharmacy Co) was injected intraperitoneally and immediately intraperitoneal injection of 3ml 0.9% NaCl was was done for hydration. In combined group of irradiation and chemotherapy, cisplatin(2mg/kg) was injected intraperitoneally immediately after irradiation(20 or 22.5 Gy).

Histopathologic examination was performed after 3 or 6 months. At least one sham-operated rat(no

Table 1. Experimental Groups and Survival Rate

Duration	No. of rats		Survived rats(%)	
	3 mo	6 mo	3 mo	6 mo
Control group	2	2	2(100)	2(100)
RT alone groups				
20Gy	5	6	5(100)	2(33.3)
22.5Gy	5	6	3(60)	0(0)
Combined RT & CT groups				
20Gy＋CT	5	5	0(0)	1(20)
22.5Gy＋CT	5	5	0(0)	1(20)
CT alone groups				
2mg/kg	5	6	5(100)	6(100)
4mg/kg	6	0	6(100)	0
8mg/kg	4	0	4(100)	0

irradiation or chemotherapy took place) was included as a control with each experimental group.

2. Tissue preparation for light microsopic examination and immunohistochemical method

Each rat was anesthetized and the brain was fixed with transcardiac infusion of 4% paraformaldehyde following perfusion with isotonic saline to remove blood from the cerebral vasculatures. The brains were removed and fixed in the same solution for a further 24 hours. Coronal sections of the supratentorial portion of each brain were taken and embedded in paraffin. Routine sections were stained with hematoxylin-eosin(H-E) and luxol fast blue(LFB) to demonstrate changes of myelin. Immunohistochemical study with anti-glial fibrillary acidic protein(GFAP) antibody was performed to evaluate the reactive changes of astrocytes.

The immunohistochemical reaction with anti-GFAP antibody was accomplished using peroxidase-antiperoxidase method as described with some modification.[11] Briefly, each deparaffinized 5-μm coronal section was reacted with a primary antiserum for 60 min before reaction with the peroxidase-antiperoxidase complex by LSAB kit from DAKO(Santa Babara, CA, USA). The antiserum for GFAP from human brain, raised in mouse, was purchased from Dako(Glostrup, Denmark) and diluted to 1:100. The peroxidase reaction was carried by incubation with link antibody and streptavidin for 20 min, respectively, and subsequently with AEC(3-aminoethyl 9-carbasol). The sections were counterstained with Meyer's hematoxylin to visualize cell nuclei. A coronal section was incubated with nonimmunized serum from the same species used to raise primary antiserum.

3. Histopathologic examination

The histopathological analysis was performed in anatomical regions of interest, which has been known as main target of irradiation in the brain, such as white matter, blood vessels in choroid plexus in lateral ventricles, and subependymal primitive cell layers. The changes of choroid plexus and basal ganglia such as epithelial cell vacuolation, perivascular edema, and vascular dilatation were graded 0 to +++ where 0 was absence of changes and +, ++, and +++ were mild, moderate, or marked severity, respectively. Vascular endothelial cell reduction and hypertrophy, vascular wall thickening, and other findings were evaulated as presence(+) or absence(−). GFAP immunoreactions(IR) were graded + to +++ as mild, moderate, and marked positive reaction. The subependymal primitive cell layers were evaluted for degree of mitosis and loss of cells with reactive glial proliferation. The hippocampi and frontoparietal cerebral cortex were also evaluated for the presence of degenerated neurons or other pathological changes.

4. Electron microscopic examination

Electron microscopic examination was performed on 6 rats, consisting of 2 control rats and experimental groups at 6 months, which were a rat with 20 Gy RT, a rat with 20 Gy RT and 2mg/ kg CT, a rat with 22.5 Gy RT and 2mg/kg CT, and a rat with 2mg/kg CT.

Sections from gray and white matter as well as basal ganglia were post-fixed in 0.1% OsO4 and embedded in epon. Light microscopic examination was performed on semi-thin sections with toluidine blue stain before electron microscopic examination. Selected ultra-thin sections were stained with uranyl acetate and lead citrate and examined by Hitachi-500 transmission electron microscope.

RESULTS

1. Survival rates

The survival rates of each experimental groups are summarized in Table 1. The rats with 20 Gy RT showed 100% survival rate at 3 months compared to 33.3% at 6 months. The rats with 22.5 Gy RT showed 60% and 0% at 3 and 6 months, respectively. All the rats with different dosages(2, 4, 8mg/kg) of CT group survived at 3 months but, only 2mg/kg CT group of rats were survived at 6 months. Combined RT and CT groups showed 0% and 20% at 3 and 6 months, respectively in both 20 Gy and 22.5 Gy RT groups.

2. Weight gain

The average body weight of experimental groups

are summarized in Table 2. With 20 Gy irradiation, average weight gain at 3 months was 50gms compared to 120-150gm increase in control rats. With 22.5 Gy irradiation, weight gain at 3 months was poor. Average weights gain were similar in rats with different dosage of CT at 3 and 6 m

onths.

3. Histopathological findings of rats with cisplatin injection

The histopathological findings depending on the dosage of cisplatin and examination time are summarized in Table 3. The rats belong to same experimental conditions showed similar histopathological findings each other. The discernible histopathological changes were changes of choroid plexus such as epithelial vacuolation, vascular dilatation and thickening, endothelial cell reduction and haphazardly scattered focal necrosis involving gray and white matter.

At 3 months post-CT, epithelial cell vacuolation, perivascular edema, and vascular dilatation in the choroid plexus were marked in rats with 2mg/kg CT compared to moderate to mild reaction in rats with 4 or 8mg/kg CT(Fig. 1A & B). At 6 months post-CT(2mg/kg), only moderate epithelial

Table 2. Experimental Groups and Body Weight

Duration	No. of rats (survived rats)		Average body weight(gm)		
	3 mo	6 mo	0 mo	3 mo	6 mo
Control group	2(2)	2(2)	220	340	370
RT alone groups					
20 Gy	5(5)	6(2)	254	304	NA
22.5 Gy	5(3)	6(0)	250	267	—
Combined RT & CT groups					
20 Gy+CT(2mg/kg)	5(0)	5(1)	260	—	320
22.5 Gy+CT(2mg/kg)	5(0)	5(1)	NA	—	NA
CT alone groups					
2mg/kg	5(5)	6(6)	225	287	290
4mg/kg	6(6)	0	220	292	—
8mg/kg	4(4)	0	220	273	—

NA : rats were survived but weights were not available
— : no rats were survived

Table 3. Histopathological Findings of Rats with Cisplatin Injection

Condition	Control		Cisplatin dosage(mg/kg)			
			2	4	8	2
Duration(month)	3	6	3	3	3	6
Number of animals	2	2	5	6	4	6
Choroid plexus						
epithelial vacuolation	—	—	+++	++	+	++
perivascular edema	—	—	+++	++	—	—
vascular dilatation	—	—	+++	+	—	—
endothelial cell reduction	—	—	—	—	—	—
endothelial hypertrophy	—	—	—	—	—	—
vascular wall thickening	—	—	—	—	—	—
Focal necrosis	—	—	+(3)	+(2)	—	+(4)

(n) : number of rats that showed changes

vacuolation was noted with no other changes.

Focal necrosis was noted in fimbria as well as cerebellar cortex and white matter(Fig. 1C & D). With 2mg/kg CT, necrosis was confined in fimbria in 3 out of 5 rats at 3 months compared to multifocal necrosis of cerebellum and fimbria in 4 out of 6 rats at 6 months. The 2 out of 6 rats with 4mg/kg CT also showed focal necrosis in fimbria at 3 months. The rats with 8mg/kg CT did not show necrosis in all 4 rats at 3 months.

Primitive cells in subependymal plates were largely well preserved in all groups. The immunohistochemical stain with anti-GFAP antibody showed no increased reaction at 3 and 6 months, except minimal reaction around focal necrotic areas.

4. Histopa

thological findings of rats with irradiation(RT) alone or with cisplatin 2mg/kg(Table 4)

1) Changes of choroid plexus in the lateral ventricle:

The rats of each group showed similar histopathological findings in the choroid plexus each other. Epithelial cells showed mild vacuolation in 20 Gy RT group at 3 and 6 months as well as in combined 20 Gy RT and CT group at 6 months. The rats in 22.5 Gy RT group showed moderate vacuolation at 3 months(Fig. 2A). Mild perivascular edema and vascular dilatation were noted in RT groups(20 and 22.5 Gy) at 3 months. At 6 months, perivascular edema was mild in 20 Gy RT group and combined 20 Gy RT and CT groups but vascular dilatation was not noted. Vascular endothelial cells were decreased in numbers in all groups at 3 and 6 months and endothelial cell hypertrophy was noted at 6 months. Vascular wall thickening was noted only in 20 Gy RT group at 6 months(Fig. 2B). The rat with 22.5 Gy RT and CT only showed endothelial cell reduction and hypertrophy at 6 months.

2) Changes in basal ganglia

Basal ganglia showed prominent changes of blood vessels with no evidence of necrosis or demyelination(Fig. 2C). Perivascular edema was mild in 22.5 Gy RT group at 3 months and moderate in all groups at 6 months. Vascular dilatation was equivocal in 20 Gy RT group and mild in 22.5 Gy RT group at 3 months compared to moderate reaction in 20 Gy RT group at 6 months. Combined RT and

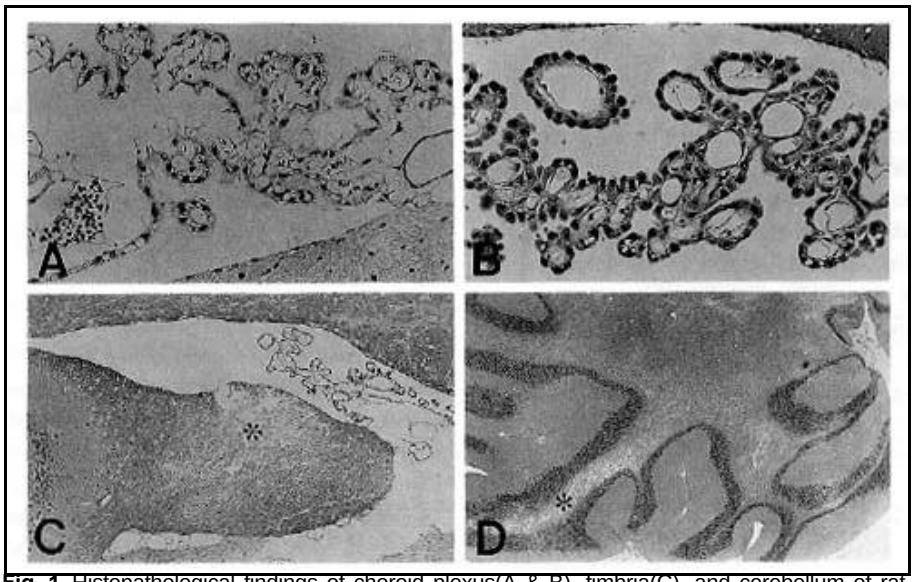

Fig. 1. Histopathological findings of choroid plexus(A & B), fimbria(C), and cerebellum of rat brain with cisplatin injection(CT). A & B: marked epithelial cell vacuolation, perivascular edema, and vascular dilatation in rat with 2mg/kg CT(A) compared to scattered vacuolation of epithelial cells in rat with 8mg/kg CT(B) at 3 month post-CT(×66, H-E). C & D: The rats with 2mg/kg CT showed focal necrosis(Asterisks) in fimbria(C, ×16, H-E) and cerebellum(D, ×5, H-E) at 3 months and 6 months post-CT, respectively.

Table 4. Histopathological Findings of Rats with Irradiation Alone or with Cisplatin

Condition	Control		20 Gy	22.5 Gy	20 Gy	20 Gy+CT	22.5 Gy+CT
Duration(month)	3	6	3	3	6	6	6
Number of animals	2	2	5	3	2	1	1
Choroid plexus							
epithelial vacuolation	−	−	+	++	+	+	−
perivascular edema	−	−	+	+	+	+	−
vascular dilatation	−	−	+	+	−	−	−
endothelial cell reduction	−	−	+	+	+	+	+
endothelial hypertrophy	−	−	−	−	+	+	+
vascular wall thickening	−	−	−	−	+	−	−
Basal ganglia							
perivascular edema	−	−	−	+	++	++	++
vascular dilatation	−	−	+/−	+	++	−	−
vascular wall thickening	−	−	−	−	+	+	+
Focal necrosis	−	−	−	−	+	+	+
Cranial nerve degeneration	−	−	−	−	+	+	+
Focal hemorrhage	−	−	−	−	+	−	−
Periventricular edema	−	−	−	−	−	+	+
GFAP positive reaction							
corpus callosum	++	++	++	++	++	+++	+++
fimbria	++	++	++	++	++	+++	++
internal capsule	++	++	++	++	++	+++	+++
caudate	+	+	+	++	++	+++	++
thalamus	+	+	+	++	++	+++	+++
globus pallidus	++	++	++	++	++	+++	+++

CT : chemotherapy(cisplatin) 2mg/kg

CT groups did not show vascular dilatation at 6 months. Vascular wall thickening was noted at 6 months in all groups.

3) Changes in subependymal plate(SEP)

In normal rats, subependymal plate about the rostral end of the lateral ventricle showed many undifferentiated primitive cells with active mitosis (Fig. 3A). GFAP showed strong positive reaction in SEP as well as in adjacent ependymal linings. The cell density in SEP was markedly decreased in number at 3 and 6 months compared to control rats and the lateral ventricles were considerably dilated(Fig. 3B).

4) Focal necrosis and other findings

Focal necrosis and cranial nerve degeneration were noted in all irr

adiated groups at 6 months (Fig. 3C & D). Multiple small focal hemorrhages were present in 20 Gy RT group at 6 months(Fig. 2D). Combined RT and CT groups showed periventricular edema at 6 months.

5) Immunohistochemical reaction with anti-GFAP antibody

Control rats showed moderate positive reaction in corpus callosum, fimbria, internal capsule, and globus pallidus compared to mild reaction in caudate nucleus and thalamus. In RT alone groups, 20 Gy RT did not increase positive reaction in corpus callosum, fimbria, internal capsule, and globus pallidus at 3 and 6 months. In caudate nucleus and thalamus, 20 Gy RT group showed similar positive reaction to control rats at 3 months (Fig. 4A) and increased positive reaction(moderate) at 6 months. The rats with 22.5 Gy RT showed increased positive(moderate) reaction at 3 months. The rat with combined 20 Gy RT and CT showed markedly increased positive reaction in all the areas at 6 months(Fig. 4B). The positive reaction was most prominent in perivascular and leptomeningeal areas. One rat with combined 22.5 Gy RT and CT showed similar(marked) reactions in most of the regions, except moderate reactions in fimbria and

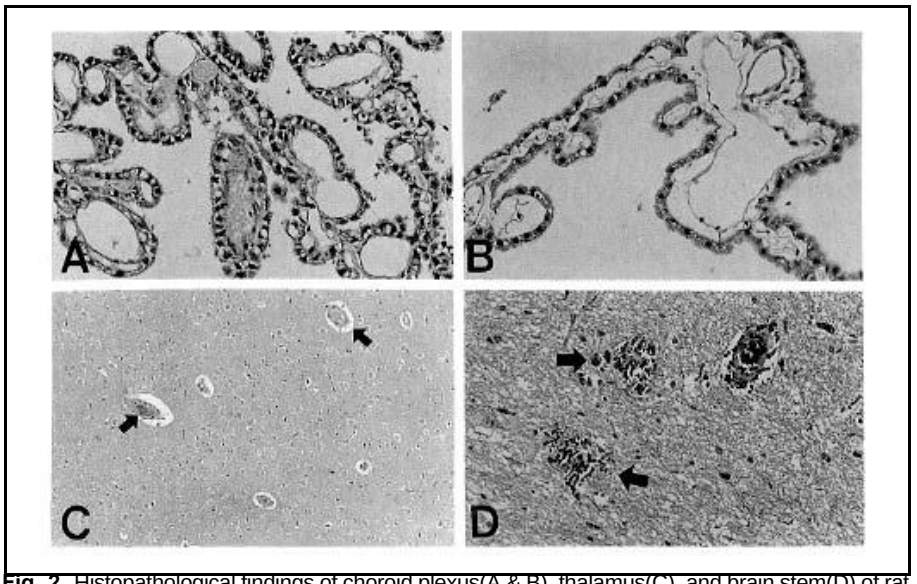

Fig. 2. Histopathological findings of choroid plexus(A & B), thalamus(C), and brain stem(D) of rat brain with irradiation(RT) alone or with cisplatin(2mg/kg) injection. A & B: The rat with 22.5 Gy RT showed moderate vacuolation of epithelial cells at 3 months post-RT(A) and rat with 20 Gy RT showed vascular wall thickening and prominent endothelial cells at 6 months post-RT(B)(×66, H-E). C & D: The rat with 20 Gy RT showed vascular wall thickening and perivascular edema(Arrows) in thalamus(C, ×33, H-E) and multiple small focal hemorrhages(Arrows) in brain stem(D, ×66. H-E) at 6 months post-RT.

caudate nucleus.

5. Light microscopic examination of semi-thin section and electron microscopic examination

A rat with CT alone showed largely well preserved neurons and axons with minimal perivascular edema. Distorted neurons are scattered in much less numbers than RT groups.

Two rats with 20 Gy RT(one with RT only and one with RT and CT) showed similar findings of blood vessels, consisting of mild to moderate vascular wall thickening and perivascular edema (Figs. 5A & 6). Distorted neurons were frequently noted as well as axonal degeneration.

A rat with 22.5 Gy RT and CT showed focal necrosis, in which blood vessels with markedly thickened wall were remaining(Fig. 5B). Distorted neurons were frequently noted as well as axonal degeneration and perivascular macrophages.

DISCUSSION and CONCLUSION

In RT alone groups, the survival rate was significantly better with 20 Gy irradiation than 22.5 Gy at 3 months(100% vs 60%) and 6 months (33.3% vs 0%). Combined group of RT and CT showed a poor survival rate, especially 22.5 Gy group. All the rats with different dosages of CT survived at 3 months but, only 2mg/kg CT group of rats were survivied at 6 months.

Average body weights were similar in rats with different dosage of CT as well as at 3 and 6 months. The body weight studied in early stage (1-10 days) by Sugimoto[12] showed a weight loss up to 14% of pretreatment level during day 4 to 10 in rats with intracarotid(IC) administration of 1.2 mg/kg and 1.5mg/kg cisplatin. In contrast, the rats with 1mg/kg did not show any significant changes in the same study. Neurological signs such as seizure, hemiparesis, lethar Gy, and coma were noted only in the group with 1.5mg/kg cisplatin. Cisplatin neurotoxicity was manifested as CT evidence of brain edema and atrophy.[13]

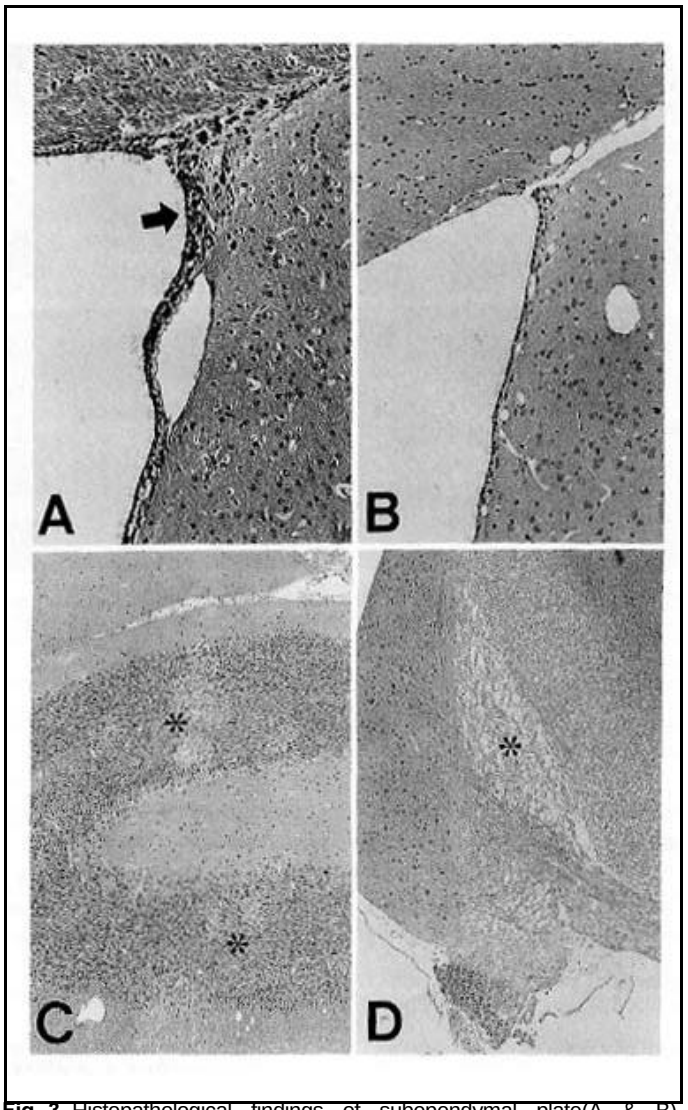

Fig. 3. Histopathological findings of subependymal plate(A & B), cerebellum (C), and cranial nerve(D) of control(A) and rats with 20 Gy RT(B-D). Actively proliferating primitive cells (Arrows) were noted in control rat(A), comparing to the absence of cells in rat with RT at 3 months post-RT(B)(×40, H-E). Multifocal necrosis(Asterisks) of cerebellum(C, ×16, H-E) and cranial nerve degeneration(Asterisks)(D, ×13, H-E) were noted at 6 months post-RT.

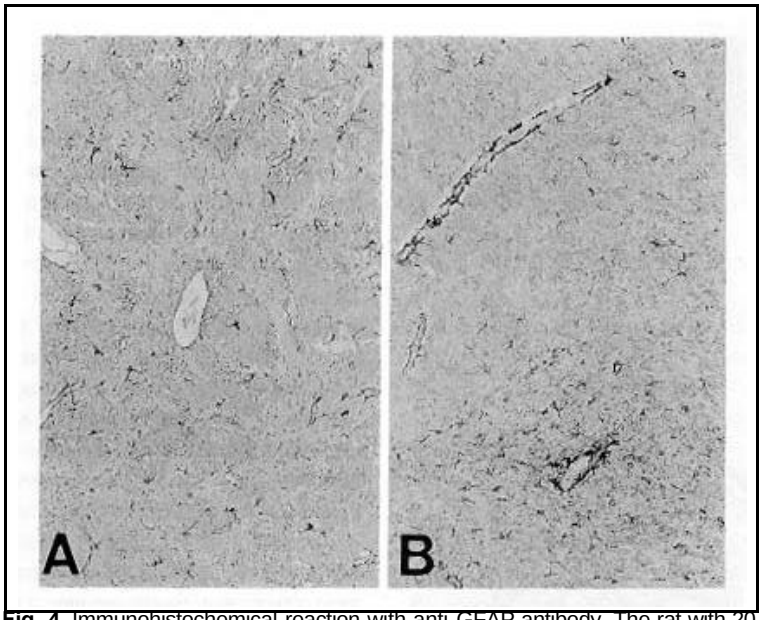

Fig. 4. Immunohistochemical reaction with anti-GFAP antibody. The rat with 20 Gy RT showed similar positive reaction to control rat at 3 month post-RT(A) and the rat with combined 20 Gy RT and CT showed markedly increased positive reaction at 6 months post-RT(B)(×33, PAP).

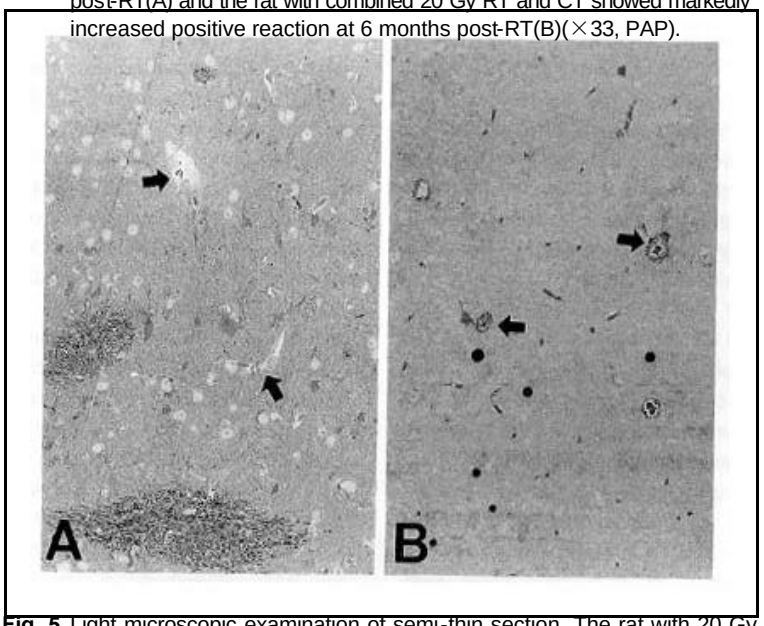

Fig. 5. Light microscopic examination of semi-thin section. The rat with 20 Gy RT showed mild to moderate vascular wall thickening and perivascular edema in basal ganglia(Arrows) at 6 months post-RT(A) and the rat with combined 22.5 Gy RT and CT showed blood vessels with markedly thickened wall(Arrows) in necrotic background at 6 months post-RT (B)(×66, Toluidine blue).

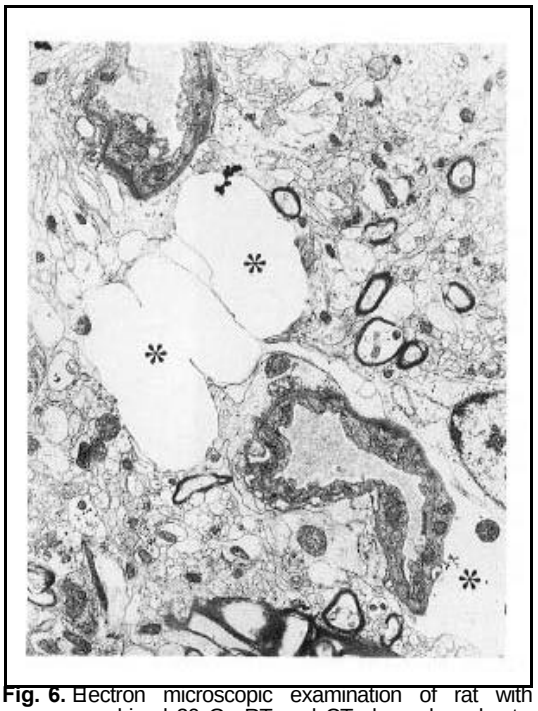

Fig. 6. Electron microscopic examination of rat with combined 20 Gy RT and CT showed moderate vascular wall thickening with perivascular edema(Asterisks) at 6 months post-RT(× 8,500).

Distribution studies of cisplatin in animals showed either a small amount of platinum in the normal brain after IV or IP injection[14, 15] but human studies showed a high accumulation of platinum or radiolabelled cisplatin in brain tumor[16, 17] and in the normal brain tissue adjacent to the tumor following IV or IC injection.

Since cisplatin passes very little through a normal blood brain barrier(BBB), the toxicity observed must be directly related to the exposure of microcirculation to cisplatin and a subsequent toxic reaction, which are first started in the endothelial cells.[8, 9, 18] Double-tracer autoradiography study in rats during 1-10 days after IC administration of cisplatin showed increased BBB permeability on ipsilateral side, which was dose-dependent and preceded the brain necrosis.[12] With 1.5mg/kg cisplatin IC administration, statistically significant BBB changes were noted in hypothalamus, auditory cortex, and caudoputamen. Local blood flow changes did not showed until brain necrosis was noted. Histologic findings observed were focal brain necrosis associated with or without microhemorrhages during 4-10 days, although it occurred haphazardly and was observed not in a majority of animals in any of the groups with different dosages of cisplatin. In this study, multifocal necrosis involving fimbria and cerebellar hemispheres was noted at 3 and 6 months after IP injection of cisplatin, which seemed like more prominent with 2mg/kg injection(3/5 at 3 months and 4/6 at 6 months) than 4mg/kg(2/6) or 8mg/kg(0/4). The degree of severity in the different dosage was not propotional, but the mortality in 4 mg/kg and 8 mg/kg group were higher(6/6) than 2 mg/kg in 6 months. The explanation for this difference according to the dosage in 3 months group was not clear in this study and further studies with more numbers of rats will be necessary. The results of this study could not be compared with other previous studies because examination time and cisplatin administration method were different. The results in this study were different from the study by Kociba and Sleight[10] showing no histological changes at 1-28 days after massive dosage(12mg/kg) of cisplatin IP administration.

The histologic changes of choroid plexus after cisplatin IP injection have not been reported in the literature. In this study, choroid plexus showed most striking vacuolation in epithelial cells with perivascular edema and vascular dilatation at 3 months with 2mg/kg injection.

Irradiation-induced changes of central nervous system(CNS) include early delayed(2-13 weeks after irradiation) or late delayed(months or years after irradiation) effects. The late delayed effects consists of blood vessel changes(fibrinoid necrosis of vascular wall with vascular thrombosis and telangiectases) and white matter damages (coagulative necrosis and demyelination). Loss of reproductive ability of cells in subependymal plate(SEP) as well as vascular damages have been known as mechanism of post-irradiation white matter necrosis and irradiation encephalopathy.[19-22]

Previous study on irradiated mature rat brain during first 3 months post-irradiation[23] showed that the cell density in SEP was less than 10% of normal control at 10 days and 0% at 3 months. Hopewell and Cavanagh[20] showed dose-dependent changes in mitotic activity of SEP. With the doses of 20 Gy or less, mitotic counts showed rapid decline in the first 24 hours after irradiation and a rise to a peak at 7 days with further decline up to 14 days, followed by

gradual return by 3 months to within normal limits. They also showed dose-dependent recovery; 1 month after 2 Gy of local irradiation compared to 3 months after 20 Gy irradiation. No recovery was observed with 40 Gy irradiation with no mitotic count at 6 months after irradiation. In this study, cell density in subependymal plate was totally absent at 3 and 6 months with both 20 and 22.5 Gy RT and mitotic counts could not be performed. The different results in this study was probably due to different experimental conditions with radiation ener Gy and species of animal.

Vascular damages in radiation encephalopathy have been described as dose-limiting tissue components(Tissue Injury Unit, TIU), which consist of a dilatation of the vascular lumen, a thickening of the blood vessel wall, an enlargement of endothelial cell nuclei, and a hypertrophy of adjacent astrocytes. Those parameters contributing to "TIU" present a much stronger correlation with demyelination than with number of astrocytes, oligodendrocytes, or endothelial cells.[24] This study showed characteristic blood vessel changes in basal ganglia and choroid plexus such as moderate perivascular edema and mild vascular wall thickening as well as endothelial cell hypertrophy and redistribution, which were similar to previous reports.[25-28] Above changes as well as vascular dilatation and focal hemorrhages were more prominent at 6 months post-RT than 3 months. The rats with 22.5 Gy RT showed more perivascular edema and vascular dilatation in basal ganglia than those with 20 Gy RT at 3 months post-RT. No previous reports showed the changes of blood vssels at 3 months post-RT. At 6 months post- RT, vascular dilatation and endothelial cell enlargement were absent in rats with 20 Gy RT compared to minimal reaction in 1 and 5 out of 6 rats with 22.5 Gy and 25 Gy RT, respectively[24]. Vascular wall thickening was noted in rats with 20 Gy RT at 6 months post-RT in this study, which was more marked than previous study by Reinhold et al,[24] showing minimal reaction with 25 Gy at 6 months and first noticed reaction at 39 weeks post-RT in rats with 20 Gy RT. Hypertrophy of astrocytes, demonstrated by GFAP- positive cells in this study, was increased in thalamus and basal ganglia at post-RT 3 months of 22.5 Gy and was markedly increased in all areas at 6 months post-RT of 20 Gy. GFAP- positive cells were aggregated in perivascular and leptomeningeal areas and combination of CT and RT did not give discernible differences. Reinhold et al[24] showed no astrocyte hypertrophia at 6 months post-RT in the rats with 20 and 22.5 Gy irradiation and minimal reaction in 4 out of 6 rats with 25 Gy RT.

Multifocal coagulation necrosis of white matter was noted in the rats with RT alone or combined RT and CT at 6 months post-RT, which was similar to previous report showing necrosis of white matter after a latent interval of more than 26 weeks.[25] They showed dose-related incidence of necrosis at 39 and 52 weeks after irradiation. Other findings such as focal hemorrhages and cranial nerve degeneration in this study were similar to previous reported studies.[27, 29-32]

Epithelial cells of choroid plexus showed mild to moderate vacuolation at 3 and 6 months post-RT, which was most prominent in rats with 22.5 Gy RT at 3 months post-RT. Vacuolation of epithelial cell was the prominent changes in the irradiated mature rat brain during first 3 months post-RT.[23] Atrophy of epithelial cells were not present in this study compared to previous study showing atrophy of epithelial layer after 13 weeks with recovery by 39 weeks.[26] Consistant endothelial cell reduction at 3 and 6 months post-RT and endothelial cell hypertrophy at 6 months post-RT in this study were similar to previous reported studies.[33-36]

Enhancing effect by cisplatin on irradiation could not be evaluated in this study because only 2 rats were survived in combined group. Among histopathologcal changes, focal necrosis was the most consistently noted finding at 6 months of radiation alone, cisplatin alone and combined groups in this study. This suggests that the degree of focal necrosis might be a good histologic finding in evaluating the delayed effects of the combined therapy with cisplatin and radiation in future studies.

REFERENCES

1. **Rosenberg R,Van Camp L, Krigas T,Trosko JE, Mansour VH.** Platinum compounds. A new class of potent antitumor agent. Nature, 1969; 222:385-386
2. **Horn Y, Adam Y, Walach N, Ilian M.** Long-term remission of an advanced head and neck tumor following intra-arterial infusion with Cis-dichlorodiammineplatinum. J. Surg Oncol 1981; 18:189-192
3. **Eagan RT, Scott M.** Evaluation of prognostic factors in chemotherapy of recurrent brain tumor. J Clin Oncol 1983; 1:38-44

4. **Douple EB, Richmod RC, Rogan MB.** Therapeutic potentiation in a mouse mammary tumor and an intracerebral rat brain tumor by combined treatment with cis-dichlorodiammineplatinum(II) and radiation. J Hematol Oncol 1977; 7:585-603
5. **Grandwetter L, Rosenstock JG, Parker RJ.** Enhanced cis-platinum neurotoxicity in pediatric patients with brain tumor. J Neurol Oncol 1983; 1: 292-297
6. **Schiffer J, Walach N, Nyska A, Lushkov G, Rothman S, Pollak L.** Brain effect of intracarotid administration of cisplatinum. Neurol Res 1996; 18: 87-88
7. **Schiffer J, Walach N, Lushkov G, Nyska A, Gur R, Pollak L.** Effect of the combination of cisplatinum and radiation in the rabbit's brain. Neurol Res 1996; 18:454-456
8. **Ichimura K, Ohno K, Hoyagi M, Tamaki M, Suzuki R, Hiratowa K.** Capillary permeability in experimental rat glioma and effects of intracartoid CDDP administration on tumor drug delivery. J Neuro-Oncol 1993; 16:211-215
9. **Neuwelt EA, Glasberg M, Frenkel E, Barnett P.** Neurotoxicity of chemotherapeutic agents after blood-brain modification; neuropathological studies. Ann Neurol 1983; 14:316-324
10. **Kociba RJ, Sleight SD.** Acute toxicologic and pathologic effect of cis-diamminedichloroplatinum (NCS-119875) in the male rat. Cancer Chemotherapy Reports 1971; 55:1-8
11. **Hsu SM, Raine I.** The value of the avidin-biotinperoxidase complex in diagnostic and research patholo Gy. In : Delellis RA, ed Advances in immunochemistry. New York : Masson, 1984;31
12. **Sugimoto S, Yamamoto YL, Nagahiro S, Diskic M.** Permeability change and brain tissue damage after intracarotid administration of cisplatin studies by double-tract autoradiography in rats. J Neuro Oncol 1995; 24:229-240
13. **Kapp JP, Vance RB.** Supraopthalamic carotid infusion for recurrent glioma. Rationale,technique and preliminary results for cisplatin and BCNU. J Nuero-Oncol 1985; 3:5-11
14. **Lange RC, Spencer RP, Harder HC.** Synthesis and distribution of a radiolabelled antitumor agent : cis-diamminedichloroplatin(II). J Nucl Med 1972; 13: 328-330
15. **Litterst CL, LeRoy AF, Guarino AM.** Disposition and distribution of platinum following parenteral administration of cis-dichlrodiammineplatinum(II) to animals. Cancer Treat Rep 1979; 63:1485-1492
16. **Stewart DJ, Leavens M, Maor M, et al.** Human central nervous system distribution of cis-diamminedichlroplatinum and use as a radiosensitizer in malignant brain tumor. Cancer Research 1982; 42: 2474-2479
17. **Ginos JZ,Cooper AJL, Dhawan V, et al.** (^{13}N) cisplatin PET to asses pharmacokinets of intra-arterial versus intravenous chemotherapy for malignant brain tumors. J Nucl Med 1987; 28:1844-1852
18. **Spigelman MK, Zappula RA, Johnson J, Goldsmith SJ, Malis LI, Holland JF.** Etoposide-induced blood-brain barrier disruption. J Neurosurg 1984; 61:674-678
19. **Hopewell JW, Wright EA.** The nature of latent cerebral irradiation damage and its modification by hypertension.Br J Radiol 1970; 43:161-167
20. **Hopewell JW. Cavanagh JB.** Effects of x irradiation on the mitotic acitivity of the subependymal plate of rats. Br J Radiol 1972; 45:461-465
21. **Keyeux A.** Late modification of cephalic circulation in head x-irradiated rats. Radiat Environ Biophys 1976; 13:125-135
22. **Reinhold HS. Hopewell JW.** Late changes in the architecture of blood vessles of the rat brain after irradiation. Br J Radiol 1980; 53:693-696
23. **Koo H, Lee KJ.** Analysis of histopatholo Gy of the irradiated adult rat brain during first 3-post- irradiation months: Expression of intrinsic kinase C and the role of apoptosis in radiation encephalopathy. Ewha Medical J 1995; 18:323-333
24. **Reinhold HS, Calvo W, Hopewell JW, van den Berg AP.** Developemnt of blood vessel-related radiation damage in the fimbria of the central nervous system. Int J Radiat Oncol Biol Phys 1990; 18: 37-45
25. **Calvo W, Hopewell JW,Reinhold HS, Yeung TK.** Time-and dose-related changes in the white matter of the rat brin after single dose of X ray. Br J Radiol 1988; 61:1043-1052
26. **Calvo W, Hopewell JW, Reinhold HS, von den Berg AP, Yeung TK.** Dose-dependent and time-dependent changes in the choroid plexus of the irradiated rat brin. Brit J Radiol 1987; 60:1109-1117
27. **Martins AN, Severance RE, Henry JM, Doyle TF.** Experimental delayed radiation necrosis of the brain. Part 1 : Effect of early dexamethasone treatment. J Neurosurg 1979; 51:587-596
28. **Myers M. Rogers M, Hornsey S.** A reappraisal of the role of glial and vascular elements in the developement of white matter necrosis in irradiated rat spinal cord. Br J Cancer 1986; 53:supple(VII) 221-223
29. **Rogers M, Myers R, Jenkinson T, Hornsey S.** Histolo Gy of the irradiated rat brain in the first post-irradiation year. Br J Radiol 1982; 55:208-212
30. **Jellinger K, Sturm KW.** Delayed radiation myelopathy in the man. J Neurol Sci 1971; 14:381
31. **Caveness WF.** Patholo Gy of radiation damage to the normal brain of the monkey. Natl Canc Inst Monogr 1977; 46:57
32. **Balkemore WF, Palmer AC.** Delayed infarction of spinal cord white matter following X-irradiation . J Path 1982; 137:273
33. **Fajardo LF, Stewart JR.** Capillary injury preceeding radiation induced myocardial fibrosis. Radiolo Gy

1971; 101:425-433
34. **Caveness WF, Tanaka A, Hess KH, Kemper TL, Tso MO, Zimmerman LE.** Delayed brain swelling and functional derangement after X- irradiation of the visual cortex in the Macaco mulatta. Radiat Res 1974; 57:104-120
35. **Hirst DG, Travis EL, Demekamp J.** The response of mesenteric blood vessels to irradiation. Radiat Research 1979; 77: 259-275
36. **Hopewell JW, Campling D, Calvo W, Reinhold HS, Wilkinson JH, Yeung TK.** Vascular irradiation damage; its cellular brain and likely consequence. British Jounal Cancer 1986;53(supple VII) 181-191

= 국문 초록 =

흰쥐 뇌에 방사선조사와 Cis-diamminedichloroplatinum(II)의 효과 : 치료 후 3개월과 6개월에서의 조직학적분석

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목 적 : 흰쥐의 전뇌에 방사선조사 후 cis-diamminedichloroplatinum(II)(cisplatin)을 복강내 주입하여 cisplatin이 방사선에 의한 후기 뇌손상(3개월, 6개월)에 미치는 효과를 알기 위하여 이 연구를 시행하였다.

방법 및 재료 : 흰쥐를 방사선조사군, cisplatin군, 방사선조사와 cisplatin 병용군으로 분류하였다. 방사선조사군은 선형가속기를 사용하여 20 Gy, 22.5 Gy의 x-ray를 각각 흰쥐의 전뇌에 단일조사하였다. Cisplatin군은 2, 4, 8mg/kg을 각각 복강내에 1회 주입하였다. 병용군은 방사선조사(20 Gy, 22.5 Gy) 후 즉시 cisplatin(2mg/kg)를 복강내에 1회 주입하였다. 각 군에 따라 치료 후 3개월과 6개월에 동물을 희생시키고 뇌의 조직병리학적 검사를 시행하였다.

결 과 : Cisplatin군은 맥락총에 상피성 공포형성과 혈관주위의 부종 및 혈관의 확장이 3개월에 보였으며, 채상과 대뇌반구에 다원성 괴사가 3개월과 6개월에 나타났다. 방사선조사군과 병용군에서 뇌의 후기 방사선손상의 특징인 국소적 응고성 괴사와 혈관의 변화가 현저하게 나타났으며 현저한 혈관주위와 연수막에 성상세포의 증식은 anti-GFAP 항체검사로 확인되었다. 방사선조사에 의한 혈관변화와 성상세포의 증식이 cisplatin에 의해서 증가되지는 않았다.

결 론 : Cisplatin과 방사선조사에 의한 후기 뇌손상의 변화로 국소적 괴사가 양군에서 동시에 나타남으로서 뇌손상의 정도를 추정하는데 괴사가 지표인자로 이용될 수 있다고 본다. Cisplatin이 방사선에 의한 혈관의 변화와 성상세포의 증식에는 영향을 미치지 않았으므로, 뇌에서 cisplatin과 방사선의 상호작용을 파악하기 위해서는 향후 더 많은 실험이 필요하다.