The Combined Effect of Adriamycin and Irradiation on the Small Intestinal Villi of Mice

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In order to clarify the effect of radiation on the mouse jejunal crypt cells by combined administration of adriamycin and radiation and also to evaluate the enhancing effect of adriamycin, the authors performed this study by delivering single irradiation of 1,000 to 1,600 rad to the whole abdomen of mice by cobalt-60 teletherapy unit. In combination with adriyamycin treatment groups, the drug was administered as single dose of 10 mg/kg either 2 hours before or 4 hours after graded single dose, 900 to 1,400 rad, of irradiation. The authors studied the quantitative changes of intestinal crypt cells by microcolony survival assay technique and the morphological changes of small intestinal villi by scanning electron microscope in mice following to combined therapy with adriamycin and irradiation.

The average number of jejunal crypts per circumference was 130 ± 16 in control group. The mean lethal dose(Do) of each irradiation alone and combined therapy groups 2 hours before and 4 hours after irradiation, were 160, 170, and 170 rad in cell survival curves, respectively. The dose effect factor(DEF) of adriamycin in each groups of pre-irradiation and post-irradiation were 1.19 and 1.26, respectively. The conical shaped villi were noted on 1,200 rad in irradiation alone group and 1,000 rad in combined groups.

For the proper clinical application we must be careful of the radiation injury to small bowel when the anticancer chemotherapy and radiation therapy to the abdomen and pelvic area are used as combined therapeutic modality.

Key words: Radiation, Adriamycin, Jejunal crypt cells, Scanning electron microscopy.

INTRODUCTION

Many methods were introduced for evaluation of the radiation effect, but the cell death was commonly used as a landmark for the basic study of cancer therapy, and the relationship between the dose of radiation absorbed and cell death was called as "dose-cell survival curve" or simplely "survival curve". In vitro the survival curve was first reported by Puck and Markus(1956),[1] but it required a certain amount of faith and optimism to believe that survival curve determined with the in vitro technique could indeed be applied to clinical radiotherapy.

The first survival curve in vivo was described by Hewitt and Wilson(1959)[2] using of lymphocytic leukemia in CBA mice. After this dilution assay technique became the basis for obtaining an in vivo cell survival curve, there were established various techniques[3] studying cell survival curve after irradiation such as survival curve for mouse skin cell, spleen colony assay system,[4] jejunal crypt cell survival assay.[5]

Following development of the macrocolony survival assay of intestinal crypt with observing nodules formed in jejunal mucosa by Withders and Elkind,[6] they have made a detailed study of response of mouse jejunum called "microcolony survival assay"[5] which is counting method of regenerating

crypt cell numbers in jejunum after whole body irradiation. This method has become a popular technique for studies on radiation damage and it was also becoming a commonly used assay for studies on fractionated irradiation,[7-9] relative biological effectiveness(RBE) according to dose rate and radiation quality,[10] and combined interaction of chemotherapeutic agents and irradiation.[11-13]

Ionizing radiation produces various damage to nuclei and cytoplasmic membranes and organelles in individual epithelial cells of small intestine,[7, 14-18] and the patterns of these damage have been recently reviewed as acute and chronic effects by Berthrong and Fajardo.[19] Such above studies contributed little to an appreciation of the changes induced by irradiation in the three-dimensional structure of the intestine, and they are inaccurate as a landmark of mucosal changes as a whole.[20] It has been observed that scanning electron microscopy is a more sensitive indicator of early mucosal damage at low radiation dose levels rather than conventional quantitative crypt counting methods.[20-26]

The principal objectives of combining chemotherapy and radiotherapy are to increase local tumor control, decrease distant metastasis, and improve survival without excessively increasing normal tissue injury.[13, 27, 28] So the ultimate goal of combined treatment modality is to improve the therapeutic ratio, but this modality may increase the side effects on the surrounding healthy tissues or organs. The one of the radiosensitive organs, the small intestine, is limiting factor for curative radiation therapy during combined modality of radiation and anticancer drugs in the treatment of abdominal, pelvic, and retroperitoneal tumors.[29] So it is essential to estimate adequate radiation dose for clinical application in combined therapy with adriamycin with the aim of reducing the side-effects on the critical organ of small intestine and improving local control rate.[30]

The author performed this study to evaluate the enhancing effect of adriamycin by delivering single irradiation or combined administration of radiation and adriamycin with different time sequence, and studied the morphological changes of small intestinal villi by scanning electron microscope in mice. The aim of this experiment was to establish the basis of radiobiological data about radiosensitivity of mouse jejunal mucosa with combining modality of radiation and adriamycin.

Table 1. Experimental Schedules

Group	Radiation dose (rad)	No. of mice
Control	0	6
Irradiation only	1,000	6
	1,100	6
	1,200	6
	1,300	6
	1,400	6
	1,500	6
	1,600	6
Adriamycin (10mg/kg) 2 hours before irradiation	900	6
	1,000	6
	1,100	6
	1,200	6
	1,300	6
	1,400	6
Adriamycin (10mg/kg) 4 hours after irradiation	900	6
	1,000	6
	1,000	6
	1,100	6
	1,200	6
	1,300	6
	1,400	6

MATERIALS AND METHODS

1. Materials

Male C3H mice aged 10 to 12 weeks were used. The mice received food and water ad libitum, and were housed in room temperature. Total 120 experimental animals were divided into control group, radiation alone, and combined groups with adriamycin 2 hours before or 4 hours after irradiation. The irradiation alone group was treated with single dose of 1,000 to 1,600 rad increasing 100 rad respectively per each subgroup. The combined groups of irradiation and adriamycin administration were irradiated to the whole abdomen absorbed dose of 900 to 1,400 rad, and single dose of adriamycin was administered intraperitoneally with a concentration equal to 10 mg/kg body weight before and after irradiation respectively(Table 1).

2. Methods

1) Irradiation

The unanesthetized mice with adequate opening for ventilation were exposed to single dose whole abdominal irradiation in acrylic jig. The set-up allow-

ed 3 mice to be irradiated at a time. Radiation treatment was given with cobalt-60 teletherapy unit with a target skin distance of 50 cm and a dose rate of 210 rad/min estimated at a midplane of the mouse by shielding of thorax and femur by 5 half value layer of lead.

2) Drugs

The adriamycin was dissolved in sterile distilled water and given intraperitoneally as a single dose of 10 mg/kg at a constant volume of 0.02 ml/g of body weight 2 hours before or 4 hours after irradiation.

3) Tissue Preparation

Each experimental groups were sacrified 84 hours after treatment, a 3 cm segment of proximal jejunum 6 cm aparted from pylorus was removed. The specimen was divided into 3 pieces of transverse sections in 1 cm length, two pieces of them were rinsed in saline and fixed in a mixture of ethanol and formalin for 24 hours and stained with hematoxylin and eosin for counting regenerating crypts, the another one was prepared with saline and sucrose for scanning electron microscopy.

4) Cell Survival Analysis

According to the method of Withers and Elkind,[5] the criteria for jejunal crypt survival were 10 or more cells, each with a prominent nucleus and a small amount of cytoplasm and lying in close proximity to one another and a crowded appearance. Nonviable crypts contained no cells, or were sparsely populated by enlarged cells with prominent eosinophilic cytoplasm.

The number of regenerating crypt (x) in each transverse section was recorded since the average number of crypts per circumference of unirradiated control was 130, the proportion of crypts destroyed by irradiation(f) is (130-x)/130. The actual numbers of surviving cells per crypt should be distributed according to Poisson statistics, and the average number would be, therefore, $-\log_e f$, and the total cell survival per circumference is 130 $(-\log_e f)$ or $-130\ (\log_e (130-x)/130)$. The cell survival curves were made by linear regression analysis of least square method after plotting radiation dose and corresponding surviving cell numbers on semilogarithmic papers.

The mean lethal dose (Do) were calculated, and the combined-radiation effects were expressed by the dose effect factor (DEF) and the isodose effect ratio (IER).

The D_{10} is the radiation dose resulting in 10 surviving cells per circumference.

$$DEF = \frac{D_{10} \text{ for radiation alone}}{D_{10} \text{ for radiation + adriamycin}}$$

The IER was introduced as the $SC_{1,000\ rad}$, and it means the number of surviving cells after 1,000 rad irradiation, unlike the D_{10} it easily can be used to test for statistically significant differences.

$$IER = \frac{SC_{1,000\ rad} \text{ for radiation alone}}{SC_{1,000\ rad} \text{ for radiation + adriamycin}}$$

5) Scanning Electron Microscopy

The samples of mouse small intestine were fixed in 2% buffered glutaraldehyde at pH 7.4. Following adequate fixation, the gut was opened longitudinally along the line of the mesentery and washed out with buffer solution, then dehydrated through an ethanol series, and dried from amylacetate. Suitable portions of tissue were post-fixed in 1% osmium tetroxide for one hour, after which the tissue was pinned out on the cork. The trimmed samples were mounted on specimen carriers with conducting adhesive solution and coating with gold in a sputter coater. The tissues were examined with scanning electron microscope used in emission mode at accelerating voltages from 1.5kV to 25kV. Recording was done with 70mm FP4 film, and the micrographs of specimens from every animal were examined for the changing in the shape of villi, erectness of villi, and the surface morphology according to increased radiation dose.

RESULTS

1. Survival Curve Characteristics

1) Radiation Alone Group

The numbers of regenerating crypts were decreased according to increasing radiation doses. The surviving crypt numbers after 1,000 rad irradiation were 118 ± 6, whereas the numbers were markedly reduced to 3 ± 1 after 1,600 rad irradiation (Table 2). The cell survial curves following radiation alone is shown in Fig-1. As indicated, the Do, D_{10}, and $SC_{1,000\ rad}$ were 160 rad, 170 rad, and 317 on the cell survival curve, respectively. The relative coefficiency of curve was 0.993.

2) Adriamycin Combined Group 2 hours Before Irradiation

As compared with radiation alone group, the decreasing rate of regenerating crypts according to radiation dose increament was more significant. The regenerating crypts for 1,000 rad and 1,400 rad were 48 ± 9 and 5 ± 1 in number, respectively (table 3). The cell survival curves following combined treatment was presented in Fig-2, the Do, D_{10}, and $SC_{1,000\ rad}$ were 170 rad, 1320 rad, and 66, respectively. The relative coefficiency of survival curve was 0.917.

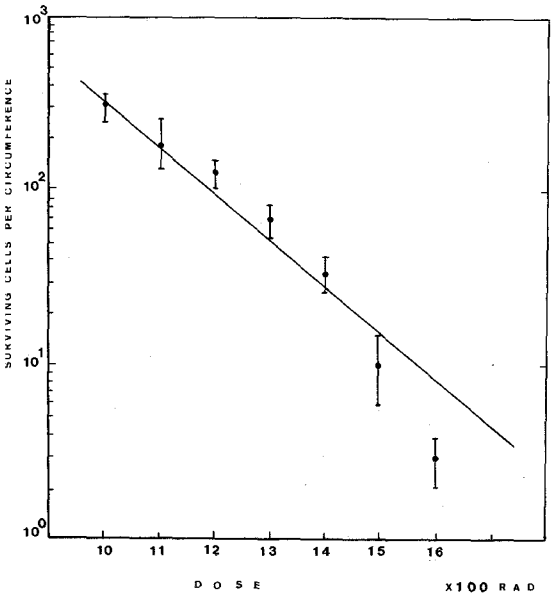

Fig. 1. Survival curve for jejunal crypt cells exposed to single dose of gamma ray.

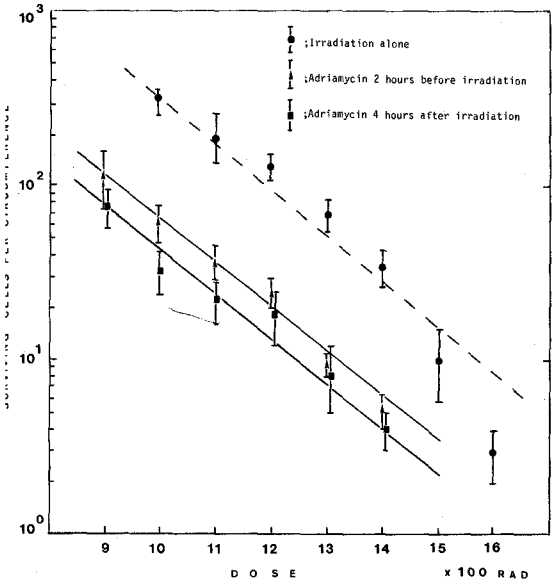

Fig. 2. Survival curves for mouse jejunal crypt cells following adriamycin given either 2 hours before or 4 hours after irradiation.

3) Adriamycin Combined Group 4 Hours After Irradiation

The numbers of regenerating crypts were remarkably diminished to 28 ± 7 and 4 ± 1 after 1,000

Table 2. Regenerating Jejunal Crypts Per Circumference in Irradiation Only Group

Radiation dose (rad)	Crypts per circumference (mean ± S.D.)
control	130 ± 16
1,000	118 ± 6
1,100	99 ± 15
1,200	83 ± 7
1,300	55 ± 8
1,400	31 ± 6
1,500	10 ± 4
1,600	3 ± 1

Table 3. Regenerating Jejunal crypts Per Circumference in ADR Injection 2 Hours Before Irradiation

Radiation dose (rad)	Crypts per circumference (mean ± S.D.)
control	130 ± 16
900	73 ± 18
1,000	48 ± 9
1,100	31 ± 6
1,200	22 ± 3
1,300	9 ± 1
1,400	5 ± 1

Table 4. Regenerating Jejunal Crypts Per Circumference in ADR Injection 4 Hours after Irradiation

Radiation dose (rad)	Crypts per circumference (mean ± S.D.)
control	130 ± 16
900	56 ± 10
1,000	28 ± 7
1,100	20 ± 5
1,200	17 ± 4
1,300	8 ± 4
1,400	4 ± 1

and 1,400 rad irradiation, respectively (Table 4). The Do, D_{10}, and $SC_{1,000\ rad}$ were 170 rad, 1,255 rad, and 42, respectively, and the relative coefficiency of survival curve was 0.925.

4) Dose Effect Factor and Isodose Effect Ratio

The DEF value estimated from survival curve for radiation alone and for adriamycin given 2 hours before or 4 hours after irradiation were 1.19 and 1.26, respectively. The IER was increased to 4.80 and 7.55, respectively, whereas the Do did no significant change (Table 5). In both cases the enhanced radiation effect, as expressed by the $SC_{1,000\ rad}$, was found to be significant ($p< 0.005$). The effect of adriamycin 4 hours after irradiation increased significantly compared with that of adriamycin 2 hours before irradiation ($p< 0.005$).

2. Scanning Electron Microscopy

1) Control Group

Although there was slight variation in the morphology of the villi over the series as a whole, the control animals displayed structural characteristics. The shape of the villi included components of apparent villus height, apparent villus width, and erectness of the villi, which were variable according to each animals and different observing sites of same specimen (Fig. 3).

2) Radiation Alone Group

The changing patterns of the villi according to increasing dose were not unifrom on the same specimen of each animals with same dose of radiation. There was a distinct irregularity of surface crease of the villi, but slight swelling and broadening at the tips of some villi were noted after 1,000 rad irradiation (Fig. 4). There were conical villi showing narrow tips and broad base and almost of villi changed into conical shape after 1,400 rad irradiation. The intestinal

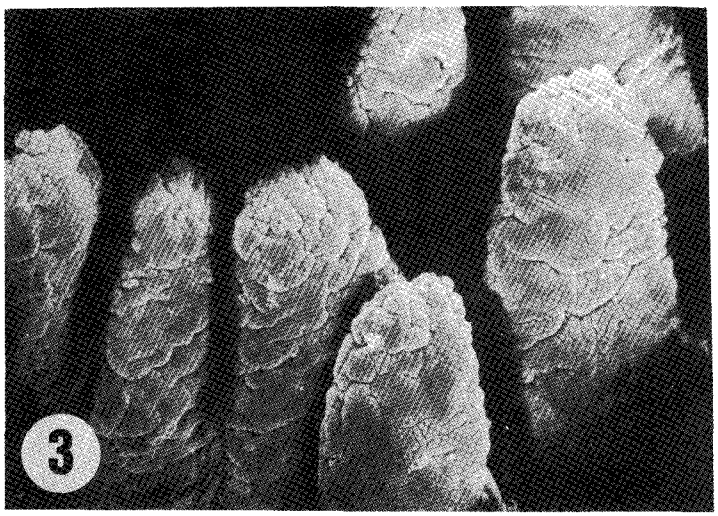

Fig. 3. SEM of normal intestinal villi. The pattern of surface crease is evident as an orderly fashion of circumferential grooves. Although there is slight variation in morphology of the villi as a whole, the erect villi show typical finger-like projections.

Table 5. Survival Curve Characteristics for Mouse Jejunal Crypt Cells Exposed to Irradiation Alone and Combined with Adriamycin

Group	Do (rad)	D_{10} (rad)	Surviving cells after 1,000 rad	DEF*	IER**
Irradiation only	160	1,570	317	—	—
ADR before RT	170	1,320	66	1.19	4.80
ADR after RT	170	1,255	42	1.26	7.55

DEF *: Dose effect factor
IER**: Isodose effect ratio

villi showed irregular and saccular swelling with loss of their configuration, and there were bending or twisting of villi and distorted into rudimentary patterns after 1,600 rad irradiation.

3) Adriamycin Combined 2 Hours Before Irradiation

The intestinal villi showed conical collapse and the base of filli were broader than tips after 1,000 rad irradiation (Fig. 5). The villi have lost their shape almost entirely and were low and irregular outline showing rudimentary collapse after 1,400 rad irradiation.

4) Adriamycin Combined 4 Hours After Irradiation

The base of the intestinal villi were broader than tips showing conical collapse but some of villi have lost their shape with low and irregular outlines in 1,000 rad irradiation group. After 1,400 rad irradiation there were multiple sacculations with diffuse adhesive changes and loss of their normal configurations(Fig. 6).

Fig. 4. SEM of intestinal villi after 1,000 rad single irradiation. There is distinct irregularity of surface crease of villi, but slight swelling and broadening at the tips of some villi are present.

Fig. 5. SEM of intestinal villi administered adriamycin 2 hours before 1,000 rad irradiation. The intestinal villi are transformed into conical shaped collapse and base of villi are broader than tips.

DISCUSSION

Shortly after the introduction of combined radiation and chemotherapy for the treatment of Wilm's tumor,[31] the clarification of damage interactions between radiation and adriamycin in mammalian cells became important because such information will allow the oncologist to design therapeutic strategies that do not compromise the efficacy of radiation on the one hand and do not result in unacceptable complications and toxicity on the others. Initially skin reactions and radiation pneumonitis were observed, but over the years augmentation of radiation effect in almost every organ has been with actinomycin-D.[31] In addition, a "recall phenomenon" has been observed with actinomycin-D, it was recurrent injury to a previously irradiated site appearing when these drugs were subsequently given, and this reaction may occur from weeks to months following irradiation.[32] After this report many authors were studied to confirm the mechanism of radiation enhancing effect on normal tissue by experimental or clinical investigation.[11-13, 28, 30, 33, 34]

The factors which influence the combined effects of chemotherapy and radiotherapy on the normal

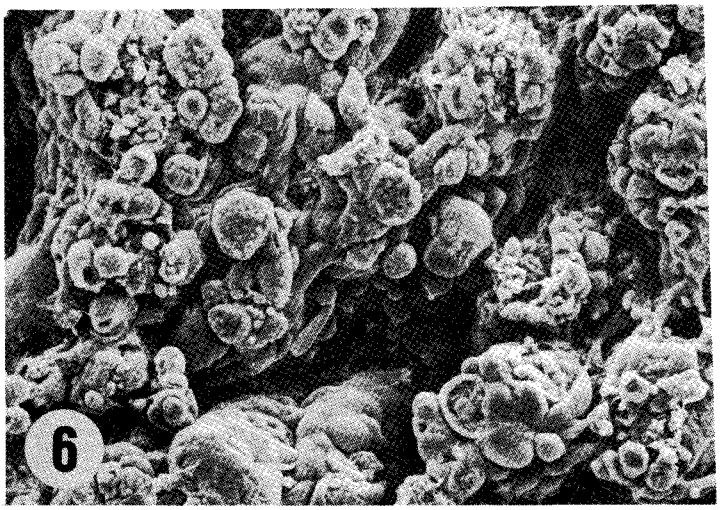

Fig. 6. SEM of intestinal villi administered adriamycin 4 hours after 1,400 rad irradiation. The intestinal villi show irregular and saccular swelling with loss of their outline showing rudimentary collapse pattern.

Table 6. Summary of Scanning Electron Microscopic Findings of Intestinal Villi according to Radiation dose in Irradiation and Combined Groups

Group	Irradiation alone				Adriamycin (10 mg/kg) before irradiation		Adriamycin (10 mg/kg) after irradiation	
Doses (rad)	1,000	1,200	1,400	1,600	1,000	1,400	1,000	1,400
Collapse pattern	Partially vertical	Vertical & conical	Conical	Rudimentary & flat	Conical	Rudimentary	Conical	Rudimentary & absent
Height & width	Mild shortening	Broad base	Short & broad	Bending & twisting	Broad base	Irregular & flatten	Irregular & low	No villi
Tips of villi	Slight narrowing	Narrowing	Mis-shaped	Lost	Narrow	Lost outline	Bending & narrow	Lost tips
Surface creasing	Mild swelling	More irregular	Severe irregular	Saccular swelling	irregular	Clumping & saccular	Irregular swelling	Adhesion & sacculation

tissues are normal tissue type,[13, 29] drug type,[29, 33] drug dose,[35, 36] administering schedule,[27] time sequence between drug and radiation administration,[27, 33, 35, 37, 38] and radiation dose rate,[39] the degree of normal tissue damage greatly depends on such as many factors. The gastrointestinal tract exhibits significant augmentation of radiation injury by the range of cancer chemotherapeutic agents and sequence and interval between drug administration and irradiation. Enhancement of radiation injury to gastrointestinal tract has been reported with a number of chemotherapeutic agents including actinomycin-D,[12, 40, 41] adriamycin,[42-45] BCNU,[46, 47] 5-FU,[48, 49] bleomycin,[11, 12, 46] especially adriamycin is representative drug frequently using for gastrointestinal cancer. But until now it was not established the exact quantitative enhancing effect of adriamycin on gastrointestinal tract when this drug was administered with radiation, so the authors chose it for experimental study.

Adriamycin is an anthracycline antibiotic that is consisted of glycone sugar and chromophore side chain.[50] In addition to its use as a single agent,[50, 51] it has also been used in combination with other drugs and with radiation,[52, 53] and it is widespreadly useful for childhood cancer, leukemias, bone and soft tissue sarcomas, breat, bladder, liver, lung, prostate, stomach, and thyroid carcinomas.[54, 55] The mode of action of the adriamycin appears to be intercalative binding to DNA with subsequent inhibition of DNA polymerase activity and RNA transcription, resulting in inhibition of the synthesis of DNA, RNA, and proteins.[56, 57] Inhibition of the synthesis of ribosomal RNA is probably an important event in the production of cytotoxicity. The toxicity manifestation of adriamycin are predominantly on cardiac and bone marrow function.[58] Apart from toxicity to adriamycin alone, a decreased tolerance of important normal tissue system to radiation has been reported. Among these tissues are bone marrow,[50] heart[59, 60], lung,[50] skin,[61, 62] and gastrointestinal tract,[11, 57, 63-66] and it also can produce chromosomal fragmentation.[67, 68]

It was possible to estimate survival rate of crypt cells by increasing single dose of adriamycin from 5 mg/kg up to 28 mg/kg, but it has been reported that above this dosage of adriamycin in mouse result in considerable gastrointestinal damage with attendant lethality within 2 days after injection of drug.[8] It was reported that the maximum tolerance dose of mouse jejunum for adriamycin is 8 mg/kg.[69] Single doses of adriamycin 5 and 10 mg/kg produce a transient reduction in the proliferative activity a of mouse jejunum, while 1 mg/kg does not cause by appreciable decrease in proliferation.[70] On cellular level, adriamycin of 10 mg/kg promptly reduced the number of mitotic figures per crypts, as fewer cells entered S phase from G1, as a result of the decreased numbers of cells passing through mitosis, the numbers of S phase cells dropped.[69-73] Thereafter the authors administered intraperitoneally 10 mg/kg of adriamycin to mouse because the proper dose was thought to be 8 to 10 mg/kg in accordance with many previous reports.

The different effect of time sequence of drug and radiation administration has been examined in gastrointestinal tract by many reporters ,[11, 58, 63, 74] but it was not established until now. It was known that the time required to diffuse into tissues and increase intracellular concentration and then metabolize is 2 hours after administration of adriamycin to mouse,[75] so the authors injected adrianmycin 2 hours before irradiation to estimate the enhancing effects of adriamycin with preirradiation treatment. The effect of the drug alone may also be estimated from their effect at administration 4 hours after irradiation as this interval exceeds the periods in which repair of sublethal radiation damage is expected to take place,[76] on the basis of this result the authors administered adriamycin 4 hours after irradiation to mouse. Von der Maase[76, 77] reported the modified microcolony assay method that is to ensure scoring of the crypt number at an equivalent crypt size, the assay time was varied according to the regeneration time in relation to the time interval and sequence of the two treatment modalities. The authors could easily observe the significant regenerating crypt both in irradiation alone and combined adriamycin and radiation groups in post-treatment 84 hours.

The mean lethal dose of jejunal crypt cells of mouse by irradiation was reported to be 100 to 140 rad,[5, 42, 78] but the authors result was 160 rad, so it was thought to be due to different species used for experimental material.

Irradiation caused epithelial hyperplasia in jejunal crypt cells and soon after irradiation cells accumulated at the G1-S border.[69] Although in vitro work suggest that cells in S-phase are more sensitive to the lethal action of adriamycin,[70] Ross et al.[69] reported that there was only slight increased in cell killing when the drug is given 3 hours after exposure as compared to 2 hours before irradiation. Epithelial hyperplasia in jejunal crypt cells after treatment with

adriamycin is found after 48 hours. Philips[11] found that more cells were killed when the drug was administered after the irradiation than when it was administered before the irradiation. It was proposed that this was caused by a modification of cell proliferation and the repair mechanism by compensatory cell regeneration of crypt cells in adriamycin administered group 2 hours before radiation than the group 4hours after irradiation.

The mechanism of radiation enhancing effect by anticancer drugs can divided into additive and synergistic effects, and the cell survival curve in vivo that shows reduced Do as ladmark of radiosensitivity and the repair dose (Dq) of sublethal damage means synergistic effect but in the case of additive effect there is little effect on the mean lethal dose of radiation survival curve that is the slope in the cell survival curve.[79] In vitro studies have shown adriamycin to be most toxic for cells in S-phase, but does not appear to substantially depress the repair of sublethal damage induced by radiation in jejunal crypt cells. So it was known that the mechanism of enhancing effect of adriamycin is caused by additive effect.[11, 29, 53] Byfield et al.[36] reported that moderate dosage of adriamycin acts as additive effect, but acts as synergistic effect in higher dosage. The possible additive effect of the combined adriamycin and radiation treatment is in accordance with observations made in other studies both on tumor cells in vitro[80] and solid tumor in vivo.[38, 78, 81] On the other hand, the authors could not conclude that this experimental results were additive effect, since there was slight difference on the mean lethal doses of radiation alone and combined adriamycin treatment groups. But this result was thought to be due to different dosage of drug, so it might be needed further study about exact mechanism of drug and radiation interactions.

If the dose of radiation required to reduce the number of intestinal micro-colonies to 10 without drug is divided by the dose of radiation required in the presence of the drug, a factor which have termed the dose effect factor (DEF) is obtained.[11-13, 29, 77] It was reported that the DEF is 1.0 to 3.3 in the case of combination of adriamycin with radiation in mouse.[11] The DEFs that adriamycin administered before and after irradiation were 1.19 and 1.26, respectively in authors' results. The results mean that combined effect of adriamycin and radiation could increase radiation effect to 26 percents, therefore it is important guideline for determining critical dose of small intestine within radiation field. Most of DEFs available in the literature from experimental studies were derived from single exposure of radiation and drug, whereas in clinical practice radiation and drug are usually delivered in fractionated regimen.[82]

The cell population of small intestine originates in the crypts of Lieberkuhn those are proliferation and stem pools and if a large enough dose of radiation over 1,000 rad is absorbed then many of the crypts are left with only but one viable cell. In mouse experiments such cells repopulate the crypts very quickly so that after 3 or 4 hours a histological section across the lumen of the irradiated intestine will show a number of regenerating crypts around the circumference.[7, 83, 84]

The quantitative technique of scanning electron microscopy provides a more sensitive indicator of early mucosal irradiation damage at low dose levels than conventional quantitative techniques.[22, 23, 25, 26] Carr et al.[20] reported that the RBE values are quoted for neutron, X- and gamma radiation given as objective scale of assessment of the effects of single or fractionated irradiation doses and as whole or partial body irradiation damage to the surface of the small intestinal villus. While radiation damage obviously involves epithelial changes, it is clear that collapse of villus may well involve the underlying stroma.[23]

It has already been suggested that different forms of villous collapse may be produced by changes in two different stromal compartments, each with possible different functions. These two compartments can be described as intravillus pegs and a peri-cryptal plate.[23] A laterally or horizontally collapsed villus has incompetent intravillous pegs and a competent pericryptal plate.[20] A vertically collapsed villus has incompetent intravillous pegs and pericryptal plate.[20] Thus more incompetence in the intravillous pegs of the vertically collapsed villus will reduce the ability of the pegs to retain the basic finger shape, there by producing a conically shaped villus. Subsequent collapse of the pericryptal plate will further lessen the support this plate gives to the villi, which will lose more height to become rudimentary.[20]

The collapse patterns of villi were variable between each same groups and according to different observing site of the same specimen in author's cases Because of this facts the degree of villous damage could not be scored by the degree of objective scales, but the patterns of villous damage were evident by increasing radiation dose. The isoeffect doses for change of conical collapse were 1,200 rad for radiation alone and 1,000 rad

for adriamycin combined treatment, respectively, so it was possible to estimate enhancing effect being about 20 percents by scanning electron microscopic findings. It was thought that the scanning electron microscopy might be valuable method for observing early change of intestinal villi by low radiation dose.

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국문초록=

방사선 조사와 Adriamycin 병용 투여가 마우스 소장에 미치는 영향에 관한 연구

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방사선 조사와 adriamycin 병용투여가 마우스 소장 소낭선세포의 방사선 감수성에 미치는 영향을 관찰하고 adriamycin의 방사선 증강효과를 측정하기 위하여 C_3H계 마우스 120마리를 cobalt-60 원격치료기로 전복부에 조사하였다.

방사선 단독조사군은 1,000 rad에서 1,600 rad까지, adriamycin 병용투여군은 900 rad에서 1,400 rad까지 각각 100 rad씩 증강시켜 조사하였고 adriamycin 10 mg/kg을 조사 2시간전과 4시간후에 각각 복강내에 주사하였다.

실험군은 84시간후에 공장을 절제하고 소낭선 측정법을 이용하여 세포생존곡선을 작성하므로써 adriamycin 병용투여시의 조사효과를 측정하였으며, 주사전자현미경(SEM)으로 소장융모의 형태변화를 관찰하여 다음과 같은 효과를 얻었다.

1) 조사군의 환상면당 소낭선수는 평균 130±16개이었다.

2) 방사선 단독조사군에서 소낭선세포의 평균 치사선량은 160 rad이었으며, 방사선조사 2시간과 4시간후 adriamycin 병용투여군은 모두 170 rad이었다.

3) 방사선조사 2시간전과 4시간후에 adriamycin 투여군의 dose effect factor(DEF)는 1.19와 1.26이었다.

4) 주사전자현미경소견에서 조사선량 증가에 따라 소장융모의 손상이 각각 다른 형태로 뚜렷이 변하였으며, conical collapse형태로 변한 것은 단독조사군의 1,200 rad와 adriamycin 병용투여군의 1,000 rad에서 각각 관찰되었다.

이상의 실험결과로 보아 마우스 소장에서 방사선과 adriamycin 병용투여시 19~26%의 유의한 조사효과 상승이 관찰되었으므로 향후 복부나 골반부에 방사선조사와 항암제를 병용하는 경우에는 방사선 감수성이 높은 장기인 소장에 대한 손상을 고려하여 임상에서 암의 방사선치료에 깊은 배려가 필요하다고 사료된다.