The Effect of Combination of Radiation with 5-Fluorouracil on Mouse Jejunal Crypt Cells

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The interaction of radiation and 5-Fluorouracil (5-FU) on mouse jejunal crypt cells was studied using the microcolony survival assay. 150mg/kg of 5-FU was injected intraperitoneally 15 minutes before irradiation and 6 hours after irradiation. Jejunal crypt cells of mouse survived more when 5-FU was given 15 minutes before irradiation than giving it 6 hours after irradiation.

The mean lethal doses (Do) of each of irradiation alone group, 5-FU injection group of 15 minutes preceding irradiation, and 5-FU injection group of 6 hours post irradiation were, 135, 135, and 114 rad respectively. The dose effect factor (DEF) of each of 5-FU injection groups of 15 minutes preceding irradiation and of 6 hours post irradiation were 1.13 and 1.27.

Key Words: Radiation, 5-FU, Mouse jejunal crypt, Interaction.

INTRODUCTION

Radiation and cancer chemotherapeutic agents are combined in order to obtain a therapeutic gain and clinically spatial cooperation is the common mode.[1,2] It is consequently of prime importance to avoid increased damage in normal tissue. Investigators have reported that various chemotherapeutic agents increased the sensitivity to irradiation.[1-10]

The combination of 5-Fluorouracil (5-FU) with radiotheraphy was used in the treatment of gastrointestinal tract malignancy,[3,4,7] and various experimental results[8-11] have shown the enhancement of radiation effect with administration of 5-FU, and most striking effect of sensitization was demonstrated when the drug was administrated following irradiation, but the exact mechanism by which 5-FU enhances the response to irradiation is poorly understood.[5,11]

The microcolony survival assay, first described by Withers and Elkind,[12] is a well established method for the study of acute radiation effect on intestinal epithelium. However modification of the assay is necessary,[10] when irradiation is combined with chemotherapeutic drugs. And the assay time has to be varied in order not to underestimate crypt survival for combined irradiation and 5-FU administration. On the basis of this modification of the microcolony survival assay, the aim of the present experiment was to evaluate the radiosensitivity of mouse jejunal mucosa and the effect of 5-FU when given before and after irradiation.

METHODS AND MATERIALS

1. Animals

Female C3H mice weighing 18-22 gm were given standard diet and water ad libitum, and were housed 5 per cage.

2. Irradiation and Drug

Unanesthetized mice with good access to air were exposed to single dose whole body irradiation in a rotating lucite chamber of Cs^{137} animal irradiator (JL Shepherd & Assoc. USA, dose rate 920 rad/min, Fig. 1.). Radiation alone group was irradiated with single dose of 1000 to 1600 rads increasing 100 rads respectively per each subgroup, each subgroup was

5 mice per dose (Table 1).

Combination groups of irradiation and 5-FU injection were irradiated to a total body absorbed dose of 800 to 1400 rads, and single injection of 5-FU was administered with a concentration equal to 150mg/kg, intraperitoneally. Post radiation and pre radiation 5-FU combination groups consisted of 35 mice respectively.

3. Assay

To ensure scoring of the crypt number at an equivalent size, the assay time varied according to the regeneration times for each radiation-drug combination as previously described by Von der Masse.[10] For radiation alone group the animals were sacrificed by cervical dislocation 90 hr after radiation and for combination group 120 hr after the last given treatment irrespectively of the drug-radiation sequence (Table 2). Surviving jejunal crypts were assayed by the microcolony technique of Withers and Elkind.[12] A 2cm segment of jejunum 6-8cm caudal to the pylorus sphincter was sectioned, and following standard histological processing, transverse sections were prepared and stained with haematoxylin and eosin. Crypt scoring criteria followed those of Withers and Elkind.[12] Approximate objective criteria for a regenerating crypt were 10 or more cells, each with a prominent nucleus and little

Table 1. Experimental Design

Group	Radiation dose (rad)	No. of mouse
Control	—	5
Radiation alone	1000	5
	1100	5
	1200	5
	1300	5
	1400	5
	1500	5
	1600	5
5-FU (Preirradiation)*	800	5
	900	5
	1000	5
	1100	5
	1200	5
	1300	5
	1400	5
5-FU (Postirradiation)**	800	5
	900	5
	1000	5
	1100	5
	1200	5
	1300	5
	1400	5

* 5-FU (150mg/kg) 15 min. before irradiation

** 5-FU (150mg/kg) 6 hr. after irradiation

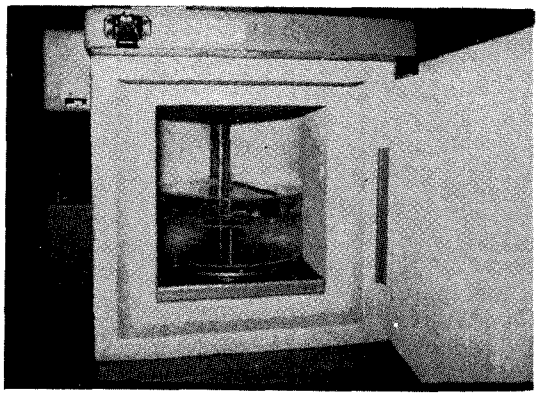

Fig. 1. Irradiation set-up with Cesium-137 animal irradiator.

Table 2. Experimental Schemes

Group				
Radiation alone (1000-1600 rad)	→	sacrifice 90 hr following radiation	→	count regenerating crypts of jejunum
5-FU (Preirradiation)	→	sacrifice 120 hr following radiation	→	"
5-FU (Postirradiation)	→	sacrifice 120 hr following injection	→	"

Table 3. Regenerating Crypts per Circumference in Radiation Alone-Group

Radiation (rad)	Crypts per circumference
1000	105±11 (S.D.)
1100	87± 9
1200	45± 8
1300	30± 4
1400	28± 8
1500	5± 3
1600	2± 1

cytoplasma, lying close together and appearing croweded. Non-viable crypts contained no cells or were sparsely populated by enlarged cells or were sparsely populated by enlarged cells with prominent eosinophil cytoplasm.

The number of regenerating crypts(x) in each transverse section was recorded. Since the average number of crypts per circumference in unirradiated controls was 140, the proportion of crypts destroyed by irradiation(f), is (140-x/140). 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 $140(-\log_e f)$ or $-140\ (\log_e (140-x/140))$.

4. Evaluation of data

The survival curve characteristics were calculated by lineal regression analysis.
The mean lethal dose (D_0), dose effect factor (DEF), and isodose effect ratio (IER) were calculated.

$$DEF = \frac{D_{10} \text{ for radiation alone}}{D_{10} \text{ for radiation } + 5\text{-FU}}$$

D_{10} being the radiation dose resulting in 10 surviving cells per circumference, and $SC_{1000rad}$ is the number of surviving cells after 1000 rads irradiation.

$$IER = \frac{SC_{1000rad} \text{ for radiation alone}}{SC_{1000rad} \text{ for radiation } + 5\text{-FU}}$$

RESULTS

The numbers of regenerating crypts per circumference of the mice of radiation alone group exposed to γ-ray of 1000 to 1600 rads seen in histological sections 90 hr after irradiation is decreased below the control value of 140 (Fig. 2, 3 and Table 3). By determining the proportion of surviving crypts

Table 4. Regenerating Crypts per Circumference in 5-FU Injection 6 hours after Irradiation Group

Radiation (rad)	Crypts per circumference
800	98±9 (S.D.)
900	70±5
1000	20±2
1100	14±2
1200	5±1
1300	2±0.5
1400	1±0.3

Table 5. Regenerating Crypts per Circumference in 5-FU Injection 15 min before Irradiation Group

Radiation (rad)	Crypts per circumference
800	120±10 (S.D.)
900	100± 8
1000	86± 6
1100	35± 3
1200	15± 3
1300	11± 2
1400	4± 1

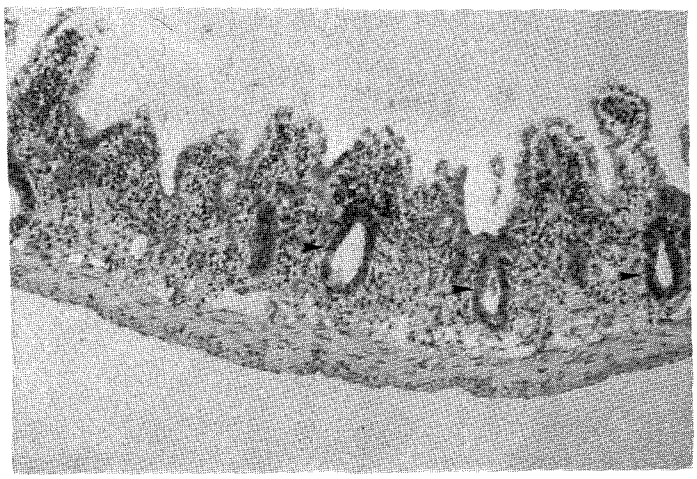

Fig. 2. Transverse section of mouse jejunum, 90 hours after 1400 rad irradiation showing regenerating crypts (arrow).

after each of a subgroups of graded doses, the survival curve for stem cells constituting the crypts was made (Fig. 4), and the value of D_0 was 135 rads.

The number of crypts per circumference observed in post irradiation and pre irradiation 5-FU injection groups are shown in Table 4, and 5. There is a marked dependence on the time of 5-FU administration, and a more pronounced crypt cell killing effect was observed when 5-FU was administrated 6 hr after irradiation, than 15 min before irradiation. Survival curves for radiation alone and for 5-FU given 15 min before or 6 hr after irradiation are presented in Fig. 5. The IER increased 3.3 to 7.84, respectively, where as the D_0 value of the pre irradiation group did not change, but the D_0 of the post irradiation group decreased to 114 rads.

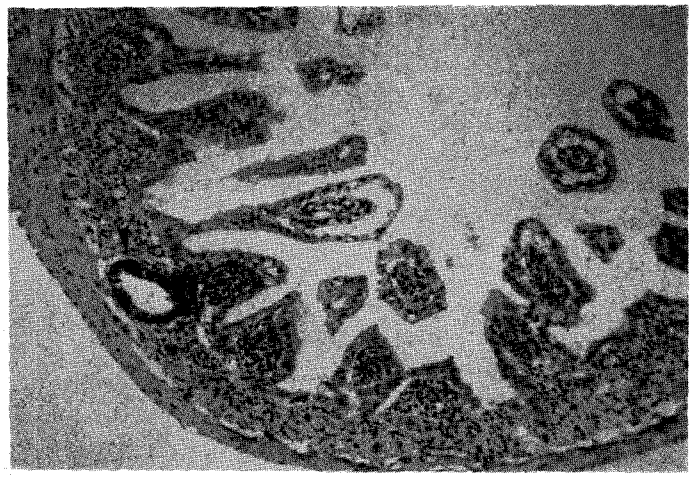

Fig. 3. Transverse section of mouse jejunum, 90 hours, after 1600 rad irradiation showing one regenerating crypt (arrow).

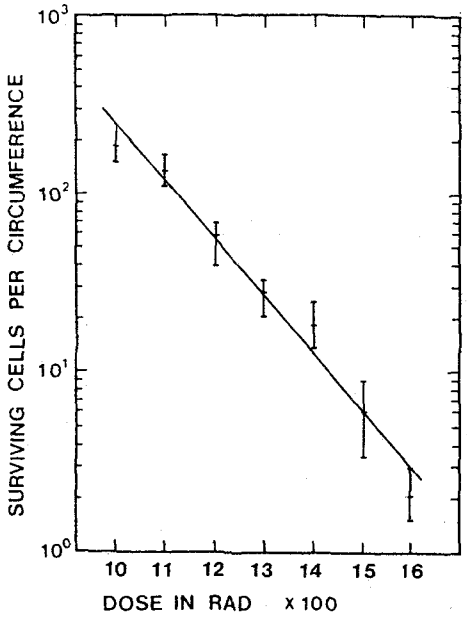

Fig. 4. Dose-survival curve for jejunal crypt cells exposed to single dose of gamma ray.

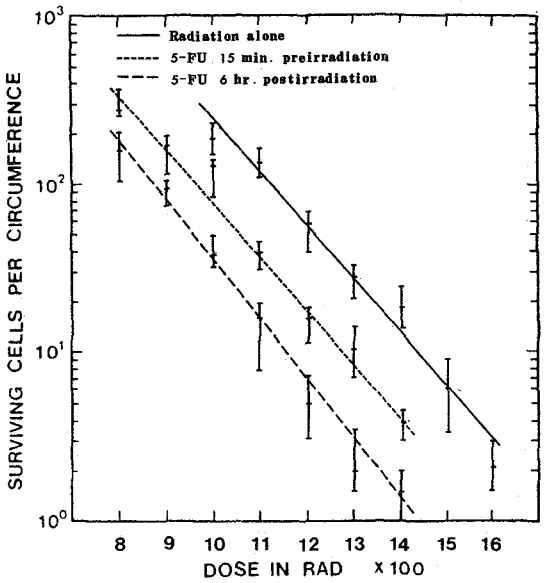

Fig. 5. Survival curves for mouse jejunal crypt cells following 5-FU given 15 minutes before and 6 hours after irradiation.

The corresponding DEF values were 1.13, and 1.27, respectively (Table 6). In both cases the enhanced radiation effect as expressed by the IER, was found to be statistically significant ($p<0.001$). The effect of 5-FU 6 hr after irradiation increased significantly compared with that of 5-FU 15 min before irradiation ($p<0.001$).

Table 6. Survival Curve Characteristics for Mouse Jejunal Cells Exposed to Radiation Alone and Combined with 5-FU

Treatment	D_0 (rad)	D_{10} (rad)	Surviving cells after 1000 rad SC 1000 rad	DEF*	IER**
Radiation alone	135	313	251	—	—
5-FU 15 min before radiation	135	313	76	1	3.30
5-FU 6hr after radiation	114	263	32	1.19	7.84

* DEF : Dose Effect Factor.
** IER : Isodose Effect Ratio.

DISCUSSION

Since the early recognition of apparant enhanced skin reaction in patients being treated for Wilms' tumor with radiation and actinomycin-D,[13] oncologists have been aware of the potential for cancer chemotherapeutic agents to interact with or potentiate the effect of ionizing radiation. The gastrointestinal tract is a rapid renewal system exhibits significant augumentation of radiation injury by a range of cancer chemotherapeutic agents.[1,2] Significant enhancement of intestinal injury in the presence of actimomycin-D, adriamycin, and 5-FU have been reported.[1,2,8-11] Quantitative data are available from laboratory experiments shows DEF's ranging from 1.1 to 1.7 of these drugs by Phillips et al.[1,2]

Radiation and cancer chemotherapeutic agents are combined in order to obtain a therapeutic gain, but to avoid unexpected complications and to obtain a more appropriate biological administration of the two treatment modalities, the effect on critical normal tissue should be studied in experimental in vivo system before clinical application of the combined treatments.[10,11]

The microcolony survival assay, first described by Withers and Elkind,[12] is a well established method for the study of acute radiation effects on intestinal tract. The assay has primarily been used to investigate the effect of radiation alone,[14-16] but it has been also applied to evaluate the effects of combined radio- and chemotherapy.[8,10,11] However modification of the assay was suggested when radiation is combined with chemotherapeutic drugs by Von der Maase.[10] Drugs may increase the regeneration time for the surviving crypts, and consequently the assay time has to be varied in order not to underestimate crypt survival for the combined treatments. On the basis of this modification,[10] authors also use this modification, and for radiation alone group the regeneration was 90 hr, when 5-FU were given assay times of regeneration was 120 hr from the last given treatment.

The halogenated pyrimidines and their nucleosides have been extensively investigated as chemotherapeutic and radiosensitizing agents. 5-FU inhibits synthesis of thymidine by blocking the enzyme thymidylate synthetase.[17] The potential for enhancement of radiation effect combined with 5-FU was demonstrated very early by Heidelberger,[18] and 5-FU has been used in numerous clinical studies in conjunction with radiation,[3,4,7] and not usually regared as a true sensitizer as its effect is mainly additive.[5]

A potentiation of the radiation effect with increased cell killing may be associated with one or both of the two radiobiological phenomena.[5,19,20] There may be a decrease in the capacity of the cells to recover from what otherwise would have been sublethal radiation damage, with a reduction in the values of quasi threshold dose (D_q) and extrapolation number (N). Alternatively, there may be a change in the slope of the exponential part of survival curve (D_0). Some potentiation effect may be due to partial age synchronization within the mitotic cycle, so that the subsequent radiation, if administered at the more sensitive part of the cycle, will be more effective as the hydroxyurea.

The in vivo cytocidal response varied with the time of exposure to the drug before or after radiation. This timing was critical, as responses ranging from less than additive to marked potentiation could be obtained. Many authors[2,8-11] report the more pronounced effect of 5-FU was seen when the drug was given after irradiation upto 8 hr. Authors also observed the more pronounced effect of 5-FU when the drug was given 6 hr after irradiation than given 15 min before irradiation (Fig. 5, Table 6). The explanation[9-11,21-23] may be that the cells transient-

ly were brought into more 5-FU sensitive condition by the irradiation, or may be associated an interference with recovery from the sublethal damage. However the exact mechanism of this remains uncertain. It has been proposed[8,24] that a radiation induced compensatory cell proliferation may increased the cell sensitivity to 5-FU. Von der Maase[10,11] suggested the enhanced cell killing effect when 5-FU given after irradiation is mainly caused by additive effect in mouse intestine. Similar to the response in normal tissue the effect of 5-FU and radiation on malignant cells was most prominent at drug administration after irradiation.[6] But Von der Maase et al[25] report that 5-FU had no effect on the radiation response neither when administered 15 min before nor 4 hr after irradiation in a mouse mammary carcinoma.

5-FU given before irradiation shows DEF value 1.13 (Table 6), similar data by Von der Maase,[11] whereas Phillips and Fu[1,2] have reported a DEF of 1.25. As previously discussed the difference in the DEF values was probably caused by the use of constant assay time in the study by Phillips and Fu[1,2] The effect when the drug was administered before radiation was interpreted as due to synchronization of the cell by 5-FU, mainly at S phase.[9] The D_0 value did not change when 5-FU given before irradiation compared with radiation alone group (Table 6) indicate the enhanced cell kill was due to an additive effect.

Despite these experimental experience, there is still no general agreement as to the proper scheduling of sequence in the use of 5-FU with radiotherapy. Although the interaction of radiation and cancer chemotherapeutic agents in the present and other studies are obtained in experimental models, it seems reasonable to assure that the interactions are complex at clinical application. Therefore, in attempt to avoid unexpected complications and to get more therapeutic gain, more investigations of drug-radiation interactions in experimental models of normal and tumor tissue are recommended.

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=국문초록=

5-Fluorouracil 투여가 마우스 공장 소낭선세포의 방사선조사 효과에 미치는 영향

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방사선조사와 5-Fluorouracil(5-FU)과의 병용시 5-FU 투여로 인한 마우스 공장 소낭선세포의 방사선 감수성에 미치는 영향과 방사선조사 효과 증강율을 측정하기 위하여 C_3H 마우스 110마리를 대상으로 동물실험용 세시움 방사선 조사기를 이용하였다. 방사선조사 단독시행군은 1,000~1,600 rad를, 5-FU와 병용군은 800~1,400 rad의 방사선조사와 복강내 5-FU 투여를 병용하였다. 방사선조사 단독시행군은 조사후 90시간 후에, 병용요법군은 120시간 후에 마우스 공장을 횡절단하여 마우스 공장 소낭선 측정법을 이용하여 평균치사선량과, 5-FU 주입이 공장 소낭선세포 생존에 미치는 dose effect factor(DEF)를 측정하였으며, 결과는 다음과 같다.

1. 방사선조사 단독시행군, 방사선조사 15분전 5-FU 주입군, 방사선조사 6시간후에 5-FU 주입군의 평균치사선량(Do)은 각각 135, 135, 114 rad 였다.

2. 방사선조사 단독시행군에 비하여 방사선조사 15분전 5-FU 주입군과, 방사선조사 6시간후에 5-FU 주입군의 DEF는 각각 1.13, 1.27이였다.