Radioprotective Effects of Recombinant Human Epidermal Growth Factor (rhEGF) in C3H/HeJ mice

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Radioprotective Effects of Recombinant Human Epidermal Growth Factor(rhEGF) in C3H/HeJ mice

Directed by Professor Jinsil Seong

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학위과정 동안 많은 것을 배우고 마무리 할 수 있도록 도와주신 여러 선생님들과 가족들에게 감사의 마음을 전합니다. 특히 한없이 부족한 저에게 기회를 주시고 또 아낌없는 가르침과 참 진리를 몸소 보여주신 성진실 지도 교수님께 깊이 감사드립니다. 또한 바쁘신 와중에 학위 논문에 세심한 조언으로 자문을 해주신 김동구 교수님, 김종선 교수님, 이윤실 교수님 조남훈 교수님께도 깊이 감사드립니다.

실험실 생활에 있어 충고와 세심한 배려를 아끼지 않았던 안정희 선생님, 원우씨 , 산부인과의 강명화 선생님, 소화기 내과의 숙인이 등 오랜시간 동안 힘들지만 즐거운 실험실 생활을 하며 정을 나눈 많은 식구들에게 다시 한번 감사의 마음을 전합니다. 동물실험에 도움을 주신 김형관 선생님, 수의사 선생님들, 그리고 방사선 실험을 도와주신 여러 선생님에게 깊은 감사를 드립니다.

늘 관심과 사랑으로 지켜봐 주신 식구들, 특히 저의 학위논문을 위해 헌신적으로 뒷바라지 해준 신랑, 항상 같이 있어주지 못해서 미안한 마음을 갖고 있었던 소중한 아들 효민이, 마지막으로 항상 저를 위해서 새벽기도를 하루도 빠짐없이 다니시는 저의 어머니에게 이 작은 성과를 바칩니다.

2007년 12월

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ABSTRACT

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Object

In order to investigate radioprotective effects of recombinant human epidermal growth factor (rhEGF) on radiation-induced mucosal damage in C3H/HeJ mice.

Materials and Methods

The radiation damage model was established that C3H/HeJ mice exposed to a single dose whole body irradiation of 8 Gy, 10 Gy. The groups of treatment model were divided into 4 groups: control, 10 Gy irradiation alone group, rhEGFalone group, and combination group of rhEGF and radiation. The rhEGF was administered 100 ㎍/㎏ intraperitoneally on day 1, 2, 3 and 3.5. Histologic examination was performed with H&E stain in jejunal mucosa. Radiation-induced apoptosis

was determined in each group with the Apoptag kit: DNA terminal transferase nick-end labeling method. Tissue sections were evaluated for PCNA expression by immunohistochemical stain.

Results

In the radiation damage model, the 8 Gy irradiated groups statistically had less weight loss compared to the 10 Gy irradiated group. The number of crypt cells was greatly decreased at 24h after 10 Gy in jejunum crypt by H&E stain. Apoptosis index of jejunum crypt in 10 Gy irradiated group was significantly increased at 24h after irradiation($p<0.05$). In the treatment model, the combination group showed significantly improvement the reduction of weight loss and the number of radiation-induced apoptosis compared with 10 Gy irradiated group.

Conclusion

It is suggested that rhEGF represents an effective strategy to reduce small intestine mucosal injury of radiation treatment in murine models. By promoting mucosal repair and protecting mucosal layer, rhEGF decreased radiation-induced apoptosis. In conclusion, rhEGF administered treatment decrease apoptosis of small intestine mucosal after the radiation exposure.

Key Words: Radiation, small intestine, damage, rhEGF, apoptosis

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I. INTRODUCTION

Radiotherapy is one of the major treatment modalities for cancer. In Asia including Korea, gastrointestinal (GI) cancer is a major malignancy. However high radiosensitivities of GI organs proposes a major limitation in applying radiotherapy in GI cancers[1-3].

As a result of ionizing radiation, acute morphological changes of the intestine are observed within 24-48 hr. The villi gradually shorten in length and the total thickness of the mucosa is reduced. Consequently, malaborption of nutrients occurs and water and electrolyte transport can be markedly inhibited. These changes result in loss of fluid leading to dehydration, electrolyte depletion, and death[4-6].

The mechanisms underlying the effect of radiation on small intestine

have been reported apoptosis and necrosis have been identified as two main forms of cell death after radiation. However, mucosal injury has been mainly attributed to apoptosis of epithelial cells as evidenced by mouse model of GI syndrome following whole body irradiation[7,8].

The effects of recombinant growth hormone (rhGF) on intestine mucosal are not well defined. A variety of approaches using growth factor has been performed in preclinical model for the efficacy in alleviating radiation-induced mucosal injury. Keratinocyte growth factor (KGF) induces a variety of responses in epithelia, which involves stimulation of epithelial proliferation, modification of migration and differentiation processes. KGF plays a key role in wound healing, with a substantial increase in dermal transcription activity. Topical application has stimulated wound healing in animal models[9,10].

Epidermal growth factor (EGF) constitutes a polypeptide hormone that binds specifically to the epidermal growth factor receptor (EGFR) located ubiquitously in epithelial cells of the gastrointestinal tract as well as in other organs and tissues. The ligand-receptor interaction between EGF and EGFR results in intestinal cell migration and proliferation to repair and restore mucosal continuity[11,12]. The EGF is present in relevant concentrations in the saliva, in other biological fluids, and in the milk from bresat-feeding mothers, which can reach about 300 times the normal serum concentration[13,14].

The EGF produced by a recombinant technique is an analogue to

the natural or recombinant growth factor EGF with smilar cell proliferation propertis.

EGF plays a critical role in wound repair and healing. EGF has also been reported to have a mucoprotective potential[15-18]. Olsen, *et al* reported that the EGF receptor and EGF producing cells increase around gastric ulcer in rats[19]. Girdler, *et al* reported that EGF administration regulated the healing of ulcers in rats and humans[20,21]. In radiation-induced oral mucosal injury model, EGF has been shown to enhance the recovery[22]. These results suggest that EGF might play an import role in repair of small intestinal mucosal injury by radiation.

The purpose of the study was to investigate the radioprotective effect of rhEGF on radiation induced intestinal mucosal damage.

II. MATERIALS AND METHODS

1. Animals

The study has been reviewed and approved by the committee that oversees the ethics of research involving the use of animals and the welfare of the animals. The study involved 8-10 week old male C3H/HeJ mice that were bred in our specific pathogen-free mouse colony in the Division of Laboratory Animal Medicine, college of Medicine, Yonsei University. The temperature (22 ℃) and humidity (55%) were controlled constantly. The water (RO water) and diet (PMI) were supplied *ad libitum*. The care and use of laboratory animals in these experiments were based on the Guidelines and Regulations for Use and Care of Animals in Yonsei University.

2. Radiation and rhEGF administration

To establish a model of radiation-induced intestinal injury, C3H/HeJ mice were given two different radiation doses in their whole bodies: a single dose of 8 Gy or 10 Gy using clinical linear accelerator (Varian Co. Milpitas, CA, USA).

Using this mouse model, therapeutic effect of rhEGF was tested on radiation-induced intestinal injury. The mouse model was injected with rhEGF (100 μg/kg/day) i.p. on days 1 to 4 after 10 Gy irradiation. Recombinant human epidermal growth factor (rhEGF) (Daewoong

Pharmeceutic Co., Seoul, Republic of Korea) was administered i.p. on radiation-treated mice.

The mice were divided into four groups: control, radiation alone, rhEGF alone, and radiation plus rhEGF (combination group). In each group 10 mice were allocated. Control group received no RT. Radiation alone group received a single dose of radiation on their whole bodies. Combination group received a single dose of radiation plus rhEGF (100 ㎍/kg/day).

3. Analysis of body weight

Mice were weighted daily to determine changes in body weight over time. Mice were monitored closely for any sign of morbidity during the experiments.

4. Crypt survival

Intestinal damage was assessed using the jejunal microcolony assay of Withers and Elkind[23,24]. For the jejunal microcolony assay, mice were sacrificed 1 to 7 days after irradiation, and 2.5 cm segments of jejunum were removed and fixed in neutral buffered formalin. After embedding in paraffin, 4 ㎛ transverse sections were cut and stained with hematoxylin and eosin (H&E). The numbers of surviving crypts per transverse

histological section were counted using the criterion of at least 10 surviving cells as indicative of a surviving crypt. Crypts in five to eight circumferences per mouse were counted and averaged. Data were reported as mean ± SE.

5. Assessment of proliferating nuclear antigene (PCNA) expression and apoptosis index (A.I.)

The small intestine tissue was analyzed for morphological changes. Immunohistochemical stain was performed according to the method previously described using antibodies targeting proliferating cells nuclear antigen (PCNA) (PC 10; Dako A/S, Glostrup, Denmark)[25,26]. Antibodies were used at the dilution recommended by the manufacturer. PCNA count was scored on coded slides at 400X magnification. And then, ten fields of non-necrotic areas were selected randomly across each jejunum section and in each field proliferating cells were expressed as a percentage of 1000 nuclei. The number and position of labeled cells in the crypts of the small intestine were recorded. Only strongly labeled cells were counted. The proliferation count calculated as 100 times the number of labeled cells per crypt.

Assessment of apoptosis was performed according to the method previously described[27]. In brief, tumor samples were collected and apoptosis was assessed in tissue sections. The tumors were immediately

excised and placed in neutral buffered formalin. The tissues were embedded in paraffin blocks and 4 ㎛ sections were cut and stained with the Apoptag staining kit (Chemicone, Temecula, CA, USA). Apoptotic cells were scored on coded slides at 400X magnification according to the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling method. TUNEL-positive cells were considered apoptotic only when associated with apoptotic morphology as previously described and illustrated[28]. Ten fields of non-necrotic areas were selected randomly across each jejunum section and in each field apoptotic bodies were expressed as a percentage of 1000 nuclei. Statistical significance was assessed using Student's *t* test ($p<0.05$).

6. Treatment and tumor growth delay analysis

For tumor growth delay analysis, 4 experimental groups were set: control group, radiation alone group, rhEGF alone group, and radiation plus rhEGF group. In each group 10 mice were allocated. rhEGF was obtained from Daewoong Pharmeceutic Co (Seoul, Republic of Korea). The radiation alone group was irradiated when the tumors grew to a mean 7.5-8 mm in diameter. The tumor-bearing legs were treated with a single dose of 25 Gy using a linear accelerator (Varian Co., Milpitas, CA, USA). The drug alone group was given 100 ㎍/kg once daily intraperitoneally for 4 days when the tumors had grown to a mean 7.5-8

mm in diameter[29]. For the combination group, radiation administrated following the above method. The described therapies were combined to treat the radiation plus rhEGF group. Tumors were measured regularly for tumor growth delay after treatment. The effect of radiation on tumor growth was determined by measuring three orthogonal tumor diameters with calipers at 2-day intervals until the tumors grew to at least 12 mm in diameter. The effect of the treatment on tumor growth delay (AGD) was defined as the time in days for the tumors to reach 12 mm in the treated group minus the mean time to reach 12 mm in the untreated control group.

The enhancement factor of tumor radioresponse was obtained by dividing normalized tumor growth delay (NGD) with AGD caused by radiation. The NGD was defined as the time in days for tumors to reach 12 mm in mice treated by the combination treatment minus the time in days for tumors to reach 12 mm in the treated group by rhEGF only. Animals were closely observed for any occurrence of toxicity until the last observation day.

7. Statistical analysis

All values were expressed as mean ± SE. Statistical analysis was

performed by analysis of variance and the *t*-test. *P*-value of less than 0.05 indicated statistical significance

III. RESULTS

1. Radiation-induced small intestinal injury

1.1 Survival rate and change of body weight

All mice survived until day 4. In 10 Gy group however, one mouse died on day 5 and two mice died on day 7, yielding 11-day survival rate of 87.8 %. In 8 Gy group, two mice died on day 11, yielding 11-day survival rate of 90.5 % (Fig.1).

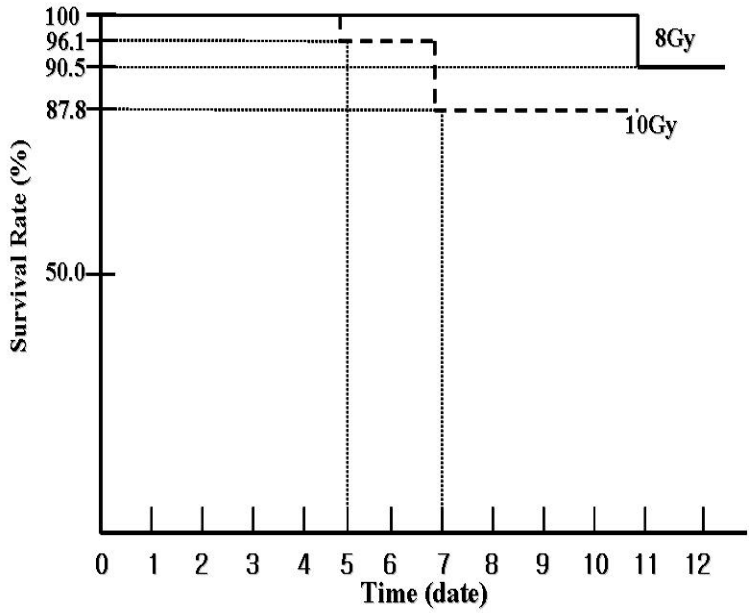


Fig.1. Survival rate after whole body radiation in C3H/HeJ mice. Values are expressed as means ± SE.

Also, 8 Gy and 10 Gy groups showed statistically significant difference in body weight after whole body irradiation ($p<0.05$) 8 Gy group revealed weight gain until day 2, and then began to show sudden decrease at day 6. On the other hand, 10 Gy group showed considerable weight loss until day 7 (Fig.2).

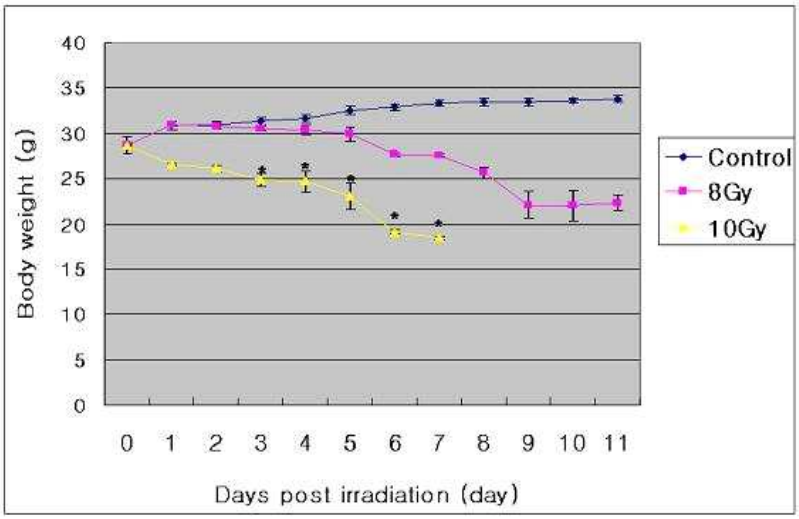


*Fig.2. Change in body weight after whole body radiation. Body weight was measured every 24 hours for 11 days (control; n=5~7, 8 Gy group; n=20~25, 10 Gy group; n=20~25). In 8 Gy irradiated group, mice body weight tended to decrease starting on day 6. But 10 Gy irradiated group, mice body weight started significant decrease on Day 3. Values are expressed as means ±SE: *p<0.05 compared control to 10 Gy and 8 Gy group.*

1.2 Number of crypt per circumference

In 8 Gy and 10 Gy group, crypts survival began to decrease on day 1. However in 8 Gy group, crypts survival increased continuously. On the other hand crypts survival decreased until day 3 and then increased until day 7 in 10 Gy group. As a result, the number of crypts per circumference was significant less in 10 Gy group than in 8 Gy group (Fig.3) ($p<0.05$).

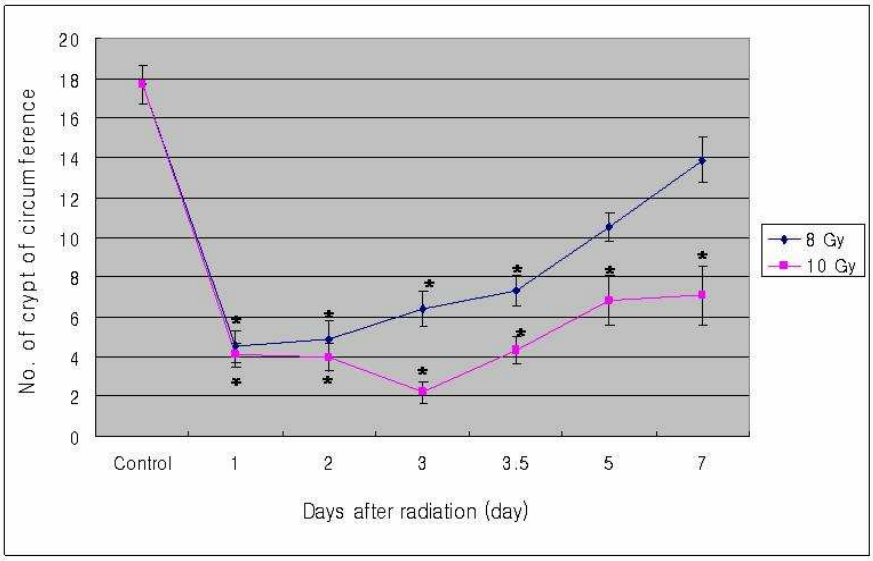


*Fig.3. Number of crypt per circumference(mm²). Jejunal crypt count after 8 Gy (◆) and 10 Gy (■) whole body radiation. Samples of the proximal jejunum were fixed in formaldehyde, and were embedded in paraffin blocks. Transverse tissue sections of the full jejunal circumference (4 ㎛ thick) were stained with H&E and scored for the number of regenerative crypts. Crypts in five to eight circumferences per mouse were counted and averaged. Vertical bars are standard deviation of mean. *p<0.05 compared control to 10 Gy and 8 Gy group.*

1.3 Apoptosis index (A.I.) in irradiated jejunum crypt

The apoptosis index was determined in the sections of the small intestine jejunum. The effect of dose was detected the segments of the intestine in mice (Fig.4).

In 8 Gy and 10 Gy groups, A.I. began to increase on day 1, but then decreased gradually day 7. Also, apoptosis dramatically increased in 10 Gy group compared with 8 Gy group. Higher than 6 fold apoptosis was shown in 10 Gy group compared with in 8 Gy group.

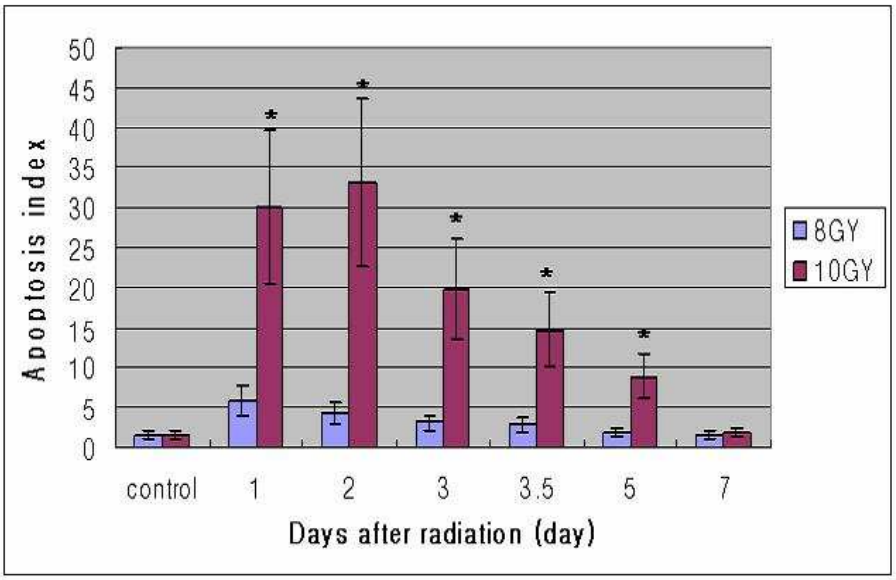


*Fig.4. Apoptosis index (A.I.) in irradiated jejunum crypt. Apoptosis cells detected by TUNEL assay show at control, 1,2,3,3.5,5 and 7 days after 8 Gy group (A) and 10 Gy group (B) whole body irradiation in crypt of C3H/HeJ mice. The apoptosis index is the number of apoptosis body per 1,000 nuclei. Vertical bars are standard deviation of mean. *p<0.05 compared control to 10 Gy and 8 Gy group.*

2. Therapeutic effect of rhEGF on radiation-induced small intestinal injury

2.1 Change of body weight

In 10 Gy group, body weight began to decrease from day 1. In 10 Gy plus rhEGF (combination group) body weight decreased from day 1 and started to recover from day 3. The difference in body weight between the 10 Gy and combination group was statistically significant ($p<0.05$). In addition, combination group showed weight gain compared with 10 Gy group beginning on day 1, whereas 10 Gy group mice showed significant decrease of body weight until day 7 (Fig. 5).

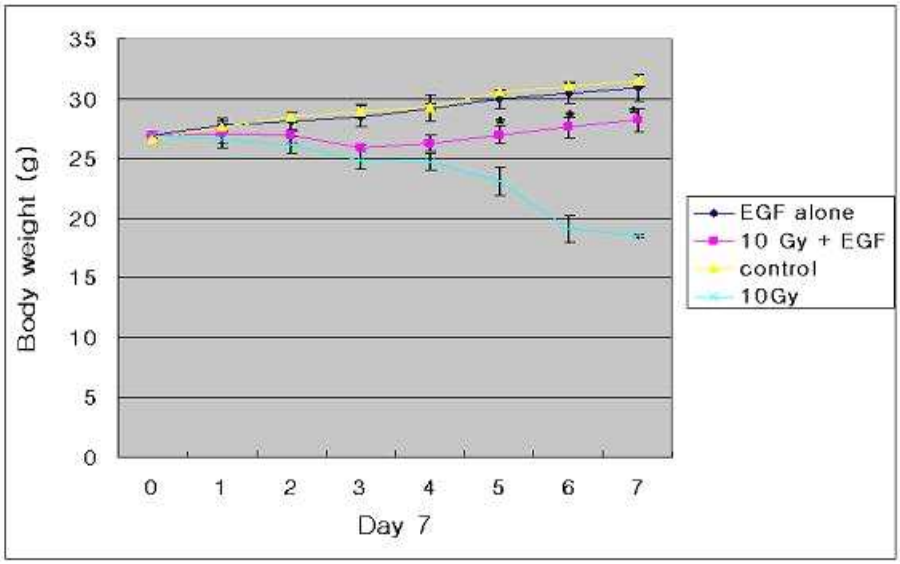


*Fig.5. Effect of EGF on mouse body weight. Change of body weight was measured every 24 hours for 7 days. Change of body weight gain after control, treatment of radiation only, rhEGF only, or combination (rhEGF plus radiation) in C3H/HeJ mice. In 10 Gy irradiated group, mouse body weight tended to decrease starting on day 3, but combination group lost significantly less weight than mouse not treated with rhEGF. *p<0.05 compared control to radiation alone and combination group. Values are expressed as means ±SE.*

2.2 A.I. and PCNA ratio in irradiated jejunum crypt

The level of apoptosis and PCNA was observed in days 5 and 7. On day 5, the A.I. and PCNA ratio significantly decreased in combination group compared with 10 Gy group ($p<0.05$) (Fig.6). Because A.I. decreased in combination group compared with 10 Gy group (Fig.6). These data suggest that rhEGF could suppress apoptosis and accelerate proliferation in radiation induced jejunal crypt injury of C3H/HeJ mice.

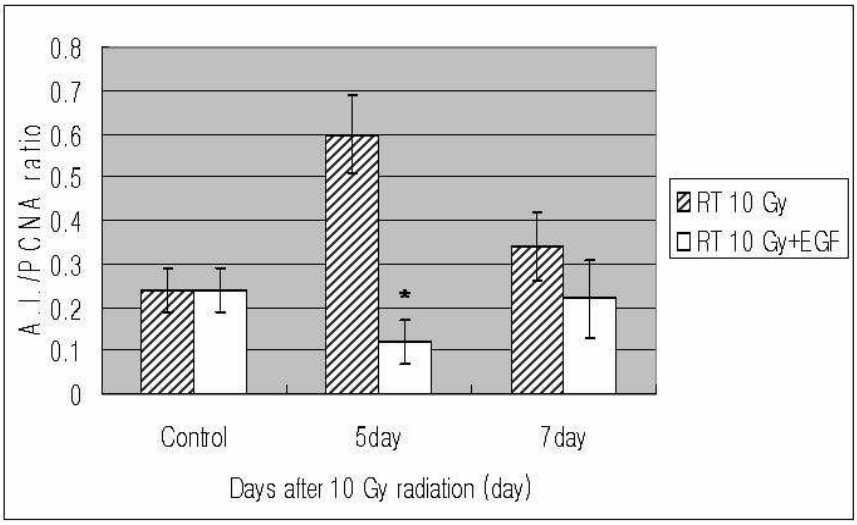


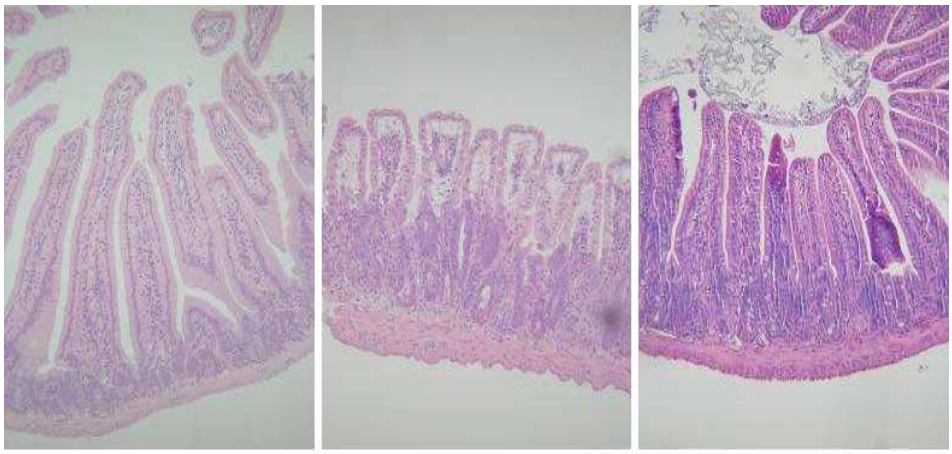
*Fig.6. A.I. and PCNA ratio in irradiated jejunum crypt. The apoptosis cells detected by TUNEL assay and PCNA index. Apoptosis showed at control, 5 and 7 days after 10 Gy whole body irradiation in crypt of C3H/HeJ mice. The apoptosis index is the number of apoptosis body per 1,000 nuclei. *p<0.05 compared radiation alone and combination group. Vertical bars are standard deviation of mean.*

2.3 Histology

To evaluate the effect of rhEGF on irradiated jejunal mucosa, the histological samples of small intestine mucosa was examined on days 3.5 and 7. Histological findings of jejunal mucosa on day 3.5 revealed difference between the radiation alone group and combination group (Fig.7. B. C). The most obvious changes in intestinal histology induced by radiation were an increase in the number of apoptotic bodies, destruction of crypt cells, necrosis of gastrointestinal epithelium and architectural changes consisting of shortening of villi as compared to rhEGF treated mice (Fig.7. B. C).

Also, the villous height decreased after irradiation. Radiation damage was maximal on day 3 but treatment with rhEGF showed decrease in the number of apoptotic bodies and increase in the number of PCNA. Moreover, villous height increased and repaired villi were observed in combination group compared with radiation alone group. However, there was no difference between the radiation alone group and combination group on day 7 (data not shown).

Our results suggest that rhEGF treatment resulted in preservation of villi, attenuation of crypt apoptosis, and enhancement of crypt proliferation.



(A) (B) (C)

Fig.7. Histopathology in jejunum after 10 Gy whole body radiation on Day 3.5 (H&E, X100). (A) Villous architecture is normal. Positive cells are noted in the full length of villi. (B) Section showing jejunum 3.5 days after 10 Gy radiation. (C) Note that the healed mucosa of mice treated with 100 μg/kg/day rhEGF. Crypts and villi are little damaged.

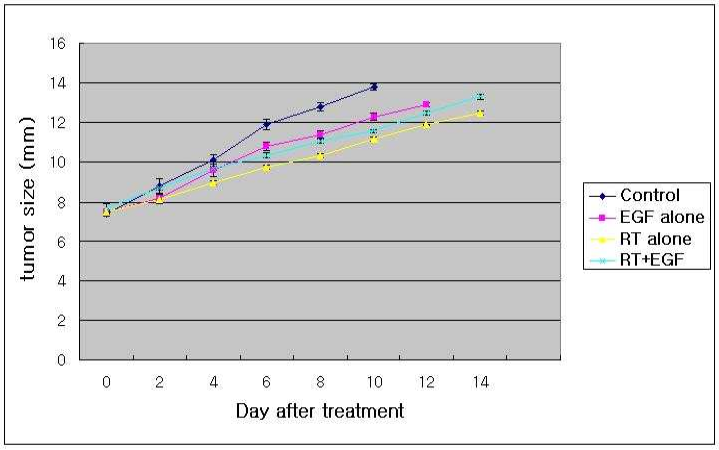
3. Tumor growth delay in rhEGF treated group

To test whether rhEGF might enhance tumor growth, tumor growth delay assay was done using murine syngeneic tumors; a radioresistant tumor, HCa-I hepatocarcinoma, (TCD 50 $\geq$ 80 Gy), a radiosensitive tumor, MCa-K mammary carcinoma (TCD 50 $\geq$ 42.9 Gy)[30].

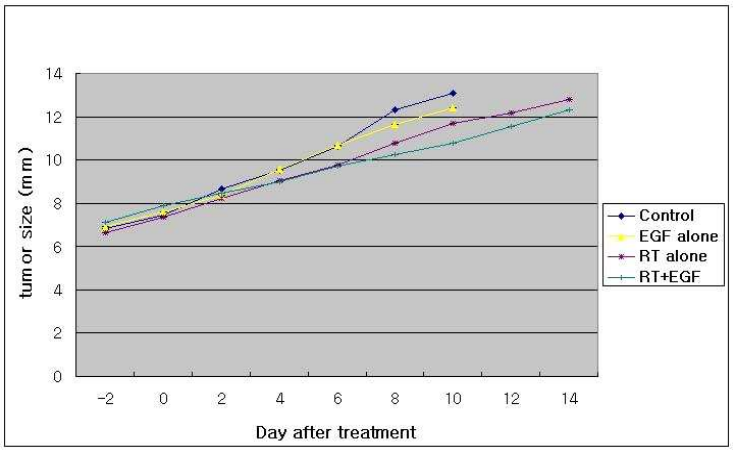
In HCa-I, the time for tumor growth from 8 to 12 mm was 11.6 days and 8.8 days in the radiation alone and the rhEGF alone group, respectively, which corresponds with an AGD of 2 days (rhEGF alone) and 4.8 days (radiation alone). When radiation was combined with rhEGF, the time for growth from 8 to 12 mm was 11.6 days and the NGD was 2.8 days. An enhancement factor was calculated as 0.6 (Fig.8.A).

In MCa-K, the time for tumor growth from 8 to 12 mm was 11.5 days and 9.2 days in the radiation alone and the rhEGF alone group, respectively, which corresponds with an AGD of 1.6 days (rhEGF alone) and 3.9 days (radiation alone). When radiation was combined with rhEGF, the time for growth from 8 to 12 mm was 11.6 days and the NGD was 3.2 days. An enhancement factor was calculated as 0.8 (Fig.8.B).

These data suggest that rhEGF did not affect the antitumor effect of radiation.



(A)



(B)

Fig.8. Tumor growth delay assay of HCa-I (A) and MCa-K (B). Tumor growth delay assay of HCa-I (A) treated with radiation (▲, rhEGF (■), combination (rhEGF plus radiation) (*) or control (◆ and and MCa-K (B) treated with radiation (*), rhEGF (▲, combination (rhEGF+radiation) (+) or control (◆. rhEGF did not affect the antitumor effect of radiation with an enhancement factor (E.F.) of 0.6 in HCa-I and 0.8 in MCa-K .

IV. DISCUSSION

Epidermal growth factor (EGF), a ~6-kDa polypeptide, induces cell proliferation, differentiation, migration. EGF is present in various body fluids and tissues, and is continuously secreted into the gastrointestinal lumen in humans by submandibular glands, mucous neck cells of the stomach, Brunner's glands of the duodenum, Paneth cells of the small intestine, and ulcer-associated cell lineage (a recently identified glandular structure induced at the site of injury). Especially, EGF and EGF family of related peptides are involved as key components in the maintenance and repair of mucosa. EGF binds to both low and high affinities sites on cells expressing the EGF receptor (EGFR). Ligand binding to the EGFR activates RTK activity leading to DNA synthesis and cell growth[31-32].

Potten, *et al*. has described the sequence of events after whole body exposure to radiation; at doses <15 Gy, surviving progenitors, albeit even a single surviving clonogen per crypt, lead to crypt recovery, identified by 3.5 days after irradiation as typical regenerative crypts. Also at doses ≥15 Gy, extensive depletion of crypt-villus units leads to mucosal denudation and animal death from the GI syndrome[33-35]. Therefore 8 and 10 Gy doses were tested for establishing radiation-induced intestinal mucosal damage model. In this study 10 Gy model was shown to be suitable since the severity of damage and its repair was dose dependent more obvious in 10 Gy compared to 8 Gy model.

In our 10 Gy model, mucosal damage was maximal on day 2 and started to recover from day 3.5, approaching to the control level on day 5 (Fig.3,4).

Several molecules have been tested and reported for alleviating mucosal damage by radiation. KGF has been reported for promising results in preventing the incidence of oral mucosal injury before irradiation[36]. rhEGF has shown suitable results in improving high incidence of oral mucositis[37].

In this study, systemic rhEGF administration alleviated loss of body weight and this effect seemed to be due to recovery of mucosal injury after radiation (Fig.5,7). We observed rhEGF to be associated with morphologic changes. In radiation alone group, on Day 3.5, crypts were diminished and villi lengths were shorten. However, rhEGF treated mice mucosa showed relatively higher level both in the number of crypt cells and the height of the villi after radiation.

Apoptosis index (A.I.) significantly decreased and PCNA index (P.I) significantly increased in combination group compared with radiation alone group on Day 5 (Fig.6). This result suggests that rhEGF protects the mucosa from radiation-induced apoptosis and accelerates proliferation, showing the possibility of rhEGF as a mucoprotectant.

We also tested a higher dose (200 μg/kg) of rhEGF to test if radiation injured mucosa showed dose dependent responses to rhEGF. From 50 to 100 μg/kg, the response was dose dependent but no

additional effect at dose of 200 μg/kg (data not shown).

In systemic administrated GFs, there might be a concern that it could either stimulate tumor growth in vivo or protect the tumor cells from radiotherapy[38]. The two tumors tested in this study involve a radioresistant one (HCa-I) and a radiosensitive one (MCa-K).(33-34) In this study, systemic rhEGF treatment did not enhance tumor growth in vivo. Showing that systemic rhEGF is protective of epithelia without enhancement of tumor growth.

In conclusion, it is suggested that rhEGF represents an effective strategy to reduce small intestine mucosal side effects of radiation treatment in murine model. By promoting mucosal repair and protecting mucosal layer, rhEGF can ameliorate intestinal mucosal injury and clinical signs such as loss of body weight.

V. CONCLUSION

It is suggested that rhEGF represents an effective strategy to reduce small intestine mucosal injury of radiation treatment in murine models. By promoting mucosal repair and protecting mucosal layer, rhEGF decreased radiation-induced apoptosis.

In conclusion, rhEGF administered treatment decrease apoptosis of small intestine mucosal after the radiation exposure.

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ABSTRACT (IN KOREAN)

Recombinant human Rpidermal Growth Factor (rhEGF)의 방사선보호작용에 대한 실험적 연구

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오 해 진

목 적: 본 연구에서는 방사선이 조사된 C3H/HeJ마우스에서 재조합표피성장인자(rhEGF)가 방사선에 의한 점막의 손상의 보호에 관여하는지를 알아보고자 하였다.

대상 및 방법: 마우스 손상 모델은 C3H/HeJ 마우스에 전신 방사선조사 (8, 10 Gy)하여 성립하였다. 실험군은 정상대조군, 10 Gy 방사선 단독그룹, rhEGF 단독투여그룹, 방사선조사와 rhEGF 투여병행군으로 각각 구분하였다. rhEGF의 투여는 1,2,3과 3.5일에 100 ㎍/kg 의 용량을 사용하여 복강에 주사 하였다. 소장점막의 병리 조직학적 검사를 위하여 H&E 염색을 시행하였다. 방사선에 의해 유도되는 세포고사는 DNA terminal transferase nick-end labeling assay 방법으로 Apoptatag kit를 사용하여 시행하였다. PCNA 발현정도를 면역조직화학염색을 통해 측정하였다.

결과: 마우스 손상모델에서, 8 Gy이 10 Gy 그룹보다 마우스의 체중이 덜 감소하였다. H&E 염색에서 10 Gy 방사선 투여군의 소낭선세포가 24시간부터 감소하였다. Apoptosis index는 10 Gy 방사선 단독투여군에서 방사선 조사 후 24시간째 의의있

게 증가되었다($p<0.05$). 방사선조사와 rhEGF 투여병행군에서는 10 Gy 방사선 단독그룹에 비하여 방사선의 의한 세포고사가 감소하였고, 마우스의 체중 감소가 향상됨을 관찰 할 수 있었다.

결론: 마우스 모델에서 rhEGF는 방사선 조사 후 소장의 점막 손상을 효과적으로 회복시켰다. 점막의 회복을 촉진시키고, 점막을 보호하는 기전에 의하여 rhEGF는 방사선에 의한 세포고사를 감소시킨다. 결과적으로 rhEGF의 처치는 방사선조사 후의 마우스 소장 점막의 세포고사를 감소시켰다.

핵심용어 : 방사선, 소장, 손상, 재조합표피성장인자, 세포고사