ORIGINAL RESEARCH

GRANULOCYTE COLONY STIMULATING FACTOR AMELIORATES RADIATION-INDUCED MORPHOLOGICAL DESTRUCTION OF INTESTINAL MUCOSA IN RATS

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ABSTRACT

Objective: We aimed to evaluate whether prophylactic administration of granulocyte colony-stimulating factor (G-CSF) has a protective effect on the morphology of irradiated intestinal mucosa.

Methods: Twenty-three animals were divided into 4 groups: saline-treated control and G-CSF-injected control groups, irradiated group with saline injection and irradiated and G-CSF administered group. G-CSF (100µg/kg/day) was given subcutaneously for four consecutive days. Twelve hours after the fourth injection, under anaesthesia a single pelvic dose of 14 Gy ionising radiation was given to the irradiated groups, while the control groups were not irradiated. On the fourth day of irradiation or sham-radiation, the rats were sacrificed and terminal ileum and rectum samples were removed for histological assessment of mucosal injury.

Results: Terminal ileum samples from both G-CSF treated and saline-treated control groups revealed minimum scores. In the saline-treated IR group, the score was significantly elevated as compared to either control group. However, G-CSF administration preceding the irradiation, reduced the severity of mucosal damage. On the other hand, the increased scores in the rectum samples of irradiated rats treated with either saline or G-CSF were not different from each other.

Conclusion: Prophylactic administration of G-CSF might reduce ionising radiation-induced mucosal destruction of the terminal ileum, but not of the rectum.

Keywords: Ionising radiation, G-CSF, Inflammation, Intestinal injury

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GRANÜLOSİT KOLONİ STİMÜLAN FAKTÖR SIÇANLARDA RADYASYONA BAĞLI GELİŞEN İNCE BARSAK MUKOZA HASARINI AZALTIR

ÖZET

Amaç: Profilaktik olarak uygulanan G-CSF'nin, radyasyona bağlı akut dönemde intestinal mukozada gelişen hasarı azaltmadaki rolü araştırıldı.

Yöntem: Wistar-Albino türü dişi sıçanlar fizyolojik tuzlu su (FTS) öntedavisi veya G-CSF öntedavisi verilen kontrol grupları ile FTS tedavisi verilmesinin ardından radyasyon uygulanan ve radyasyon öncesi G-CSF uygulanan gruplar olmak üzere 4 gruba ayrıldı. G-CSF öntedavisi alan iki grupta sıçanlara G-CSF (100µg/kg/gün) 4 gün boyunca deri altından uygulandı. Son ilaç uygulamasından 12 saat sonra sıçanların yarısına, anestezi altında pelvik alana tek doz 14 Gy radyasyon verildi. FTS veya G-CSF verilen kontrol gruplarındaki sıçanlara ise radyasyon uygulanmadı. Bütün sıçanlar ışınlamadan sonraki 4. günün başında dekapite edildi, terminal ileum ve rektumdan alınan örnekler mikroskopik olarak değerlendirildi.

Bulgular: Terminal ileum örnekleri incelendiğinde, G-CSF uygulanan kontrol grubu ile FTS uygulanmış kontrol grupları arasında anlamlı bir fark yoktu. Işınlanan gruplardan FTS verilen radyasyon grubundaki skor tüm gruplara gore anlamlı olarak daha yüksek bulundu. Buna karşılık, G-CSF uygulanan grupta bu skorun anlamlı olarak düşük olduğu gözlendi. G-CSF uygulanan ve FTS uygulanan radyasyon gruplarının rektum örnekleri arasında ise mikroskopik hasar açısından anlamlı bir değişiklik oluşmadı.

Sonuç: Deneysel çalışmamız, iyonizan radyasyon öncesi G-CSF uygulanmasının terminal ileum mukozasının morfolojik yapısının korunmasında etkili olduğunu, ancak aynı etkinin rektumda oluşmadığını göstermektedir.

Anahtar Kelimeler: İyonizan radyasyon, G-CSF, İnflamasyon, İncebarsak hasarı

INTRODUCTION

Normal tissue side effects of ionising radiation may restrict the curative doses of application on malignant tissues in routine clinical practice. Despite the advances in radiotherapy techniques and new supporting strategies, acute and consequential late side effects may not be ameliorated properly and this may affect the patients` quality of life following radiotherapy[1,2]. Since maximum tumour control is required with less toxic treatments in order to achieve a good state of the art in cancer therapy, understanding the mechanisms of radiation-induced toxicity on the gastrointestinal system and its management is crucial[3,4].

Granulocyte colony stimulating factor (G-CSF), a 20 kd cytokine producing by bone marrow, stimulates the granulopoiesis, increases the circulating polymorphonuclear leukocytes (PMN) and is currently used for different purposes in clinical practise[5]. It lessens the inflammatory reactions on the gastrointestinal mucosa in experimental inflammatory bowel disease and may play a role in the enhancement of host defence against the bacterial translocation[6]. Additionally, G-CSF was previously used for prophylaxis of sepsis in surgical intensive care patients[7,8,]. Furthermore, clinical trials approved the healing effect of G-CSF on chemo- and radiotherapy-induced oral mucositis[9]. Nevertheless, no previously published data exist testing the efficacy of G-CSF on irradiated bowel, either as a protective or therapeutic treatment modality.

The present study tested whether prophylactic administration of G-CSF may reduce radiation-induced morphological damage in the intestinal mucosae of rats that had received pelvic irradiation. For this purpose, we evaluated the extent of mucosal damage on the histological samples obtained from the terminal ileum and rectum.

MATERIAL AND METHOD

This study was approved by The Animal Care and Use Committee of our faculty. Female Wistar-Albino rats (180-280g) were used for all experiments and were kept at a constant temperature (20 ± 2 ºC) and photoperiod (12 h

light: 12 h dark). They were allowed free access to regular rat chow and water.

Twenty-three animals were divided randomly into 4 groups: control (n=5), saline-treated irradiation group (IR) (n=6), G-CSF-treated control group (n=6), and G-CSF-treated IR group (n=6), respectively. The animals were treated subcutaneously with either saline or G-CSF (100µg/kg/day; r-metHuG-CSF; Neupogen® 30MU, Roche) for four days, subcutaneously. Twelve hours after the fourth injection, the rats were exposed to ionising radiation. Initially, the animals were anaesthetised with ketamine (100mg/kg i.p.). Then, each time, 4 rats were placed on a rectangular board (40x10cm) in supine position and were irradiated covering the pelvic organs. Irradiation was performed with linear accelerator (LINAC, Saturne 42 GE) producing 6 MV photons at a focus-skin distance of 100cm. Each animal received a single dose of 14 Gy to the pelvic region. Following the radiation procedure, the animals were then returned to their home cages. On the other hand, both saline and G-CSF- treated control groups were anaesthetised and immobilised without irradiation. All animals were sacrificed on the fourth day at the 72nd hour of irradiation or sham-irradiation. Samples from the terminal ileum and rectum were removed immediately for histological analysis.

Histological Analysis

Samples were taken randomly from each animal and they were examined for histological assessment. All samples from the terminal ileum and rectum were fixed in normal 10% buffered formalin solution, dehydrated in ascending alcohol series and embedded in paraffin wax. They were stained with Haematoxylin and Eosin for general morphology. Light microscopic examination was performed by a pathologist (MGG) who was blind to the coding of the specimens. A semi-quantitative evaluation was made according to the following parameters: *a) cryptic distortion, b) epithelial denudation and erosion, c) reduction in mucus production, d) degree of polymorphonuclear cell (PNL) infiltration and e) degree of oedema in lamina propria*. Each parameter was given a score from 0 to 3, where the maximum score was 18.

Statistical analysis

Data analyses were done using the SPSS (v10.0). The group means ± SE were used and comparison of the groups was made with one-way ANOVA (the analysis of variance) followed by Tukey post-hoc test and the significance level was set at $p<0.05$.

RESULTS

In the saline-treated and G-CSF-treated control groups, normal epithelial and mucosal structure in the terminal ileum was observed. In the saline-treated control (4.60±0.60) and G-CSF-treated control (2.83±0.32) groups, the obtained scores were due to increased PNL accumulation and oedema in the lamina propria, rather than radiation-induced damage (Figure 1). However, in the saline-treated IR group, the light microscopic appearance of the terminal ileum showed severe denudation of apical surface epithelium and decrease of mucosal height, increased mucus depletion, PNL and oedema (Figure 2A). On the other hand, these effects were mild and the normal epithelial structure was maintained in the G-CSF-treated IR group (Figure 2B). The histological analysis of the samples from the saline-treated IR group revealed that the scores were the highest (8.0±1.13) as compared with the other groups ($p<0.01$), and this score was reduced back to control levels in the G-CSF-treated irradiated rats (3.80±0.73; $p=0.03$) (Figure 1).

In the rectum samples of the control groups, regular rectal mucosa was observed. On the other hand, in the saline-treated irradiated group, denudation of the rectal mucosa, accumulation of PNL, amount of mucus and oedema were comparatively less with respect to the terminal ileum samples of the same irradiated groups (data not shown). However, neither the extent of histologically apparent distortion of the morphology, nor the histological scores in the irradiated groups treated with either saline (6.50±0.67) or G-CSF (6,67±0,67), differed from each other.

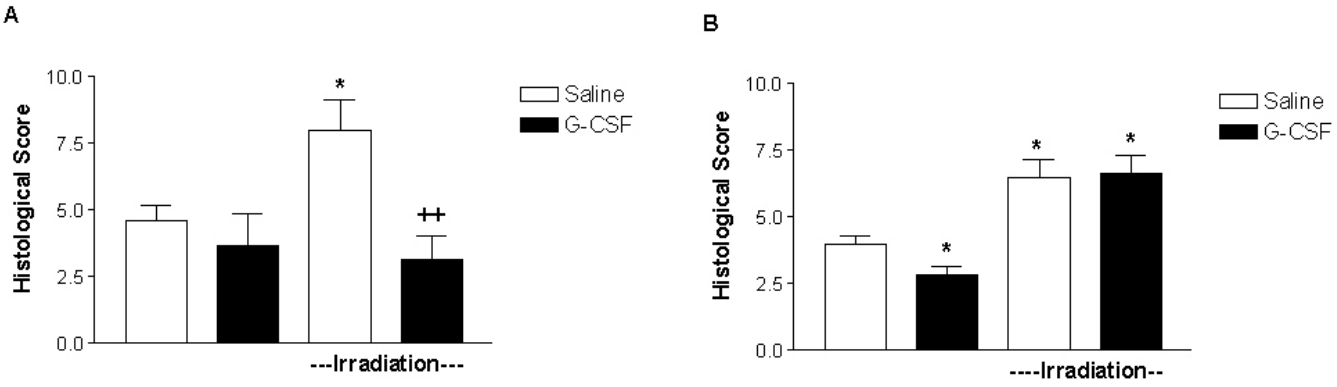

Figure 1: Histological scores of the (A) ileal and (B) rectal mucosae of saline-pretreated, G-CSF-pretreated rats that received pelvic irradiation, as compared to non-radiated rats treated with saline or G-CSF.
*p<0.05, compared to saline-treated irradiated group; ++p<0.01 compared to saline-treated irradiated group.

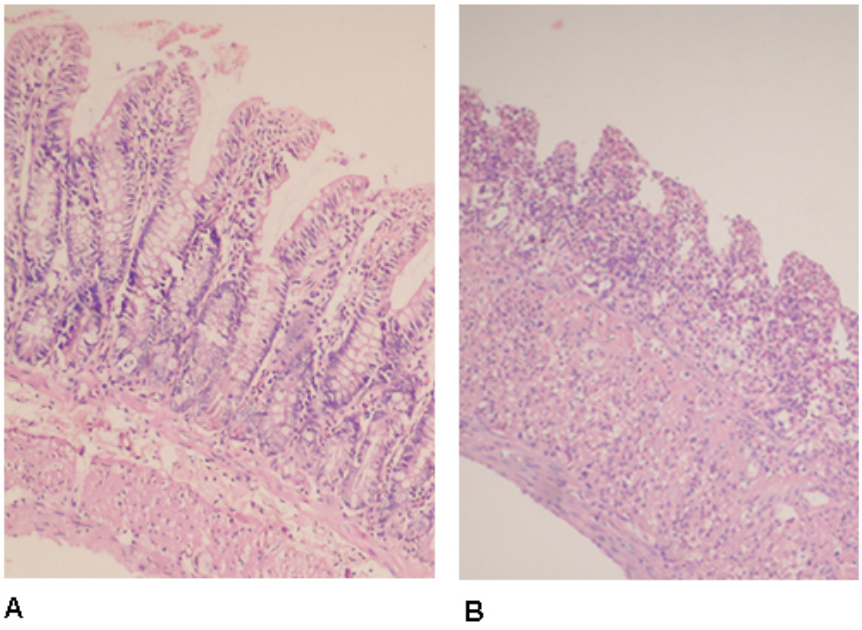

Figure 2: The micrographs showing the ileal mucosa of saline-pretreated (A) and G-CSF-pretreated (B) rats that had received pelvic irradiation.

DISCUSSION

Gastrointestinal tract injury during pelvic irradiation is observed in clinical practice with an incidence of 5 to 50 % and the symptoms show a broad spectrum like diarrhoea, rectal pain, bleeding, stricture or fistulae[3,4]. Reducing the acute side effects in radiotherapy increases the patients` quality of life and administering the normal tissue protectors during treatment is one possible

way to diminish the acute effects of radiotherapy[2,10,11]. In this experimental study, we used G-CSF, a glycoprotein that acts as a neutrophil precursor promoter, to protect the normal tissue, administering it prior to the occurrence of radiation-induced injury. We observed that G-CSF significantly decreased the radiation-induced erosion on the gastrointestinal mucosa of the terminal ileum. However, the pre-treatment had no protective effect on the rectal mucosa.

One of the probable reasons for this protective effect of G-CSF is its inhibitory effect on the inflammatory response, which is triggered by irradiation via the participation of the bacterial flora. The well-documented major early histological changes following irradiation on the intestines are inhibition of mitoses in crypts, epithelial denudation, cryptic distortion and enhanced signs of inflammation[12]. Studies showed that early responses to irradiation might be physiological and triggering inflammation through direct activation of NF-κB may begin before the structural damage occurs[13-15]. Furthermore, the studies on both irradiated and inflammatory bowel samples show similar acute epithelial barrier dysfunction and this evidence addressed that normal intestinal flora may trigger the inflammatory response on the first line mechanisms[5,13]. Even in the very early stages, only ultrastructural changes may be responsible for the injured epithelial barrier, promoting the normal flora to become pathogen, which then recruits the inflammatory mediators. Therefore, induction of neutrophil influx before bacterial translocation takes place may decrease the damage[14]. Ağalar et al.[16] have shown in a haemorrhagic shock model that prophylactic G-CSF usage may diminish the bacterial translocation incidence. Although we did not measure the amount of bacterial translocation, our results suggest that prophylactic administration of G-CSF might protect the irradiated tissue against the invasion of pathogen microorganisms and the associated inflammatory process.

Ionising radiation and tissue interactions mostly includes oxidative stress. Radiation ionises water into reactive oxygen species (ROS) like $OH^{\cdot}$ and $H^{\cdot}$ and these agents are responsible for cell and tissue damage. This is called the `indirect effect`[17]. On the other hand, neutrophils carry and release mediators such as interleukin (IL)-8 and ROS on pathogens for effective killing. Although leukocyte infiltration and accumulation in irradiated normal tissues are well described, the main consequences of neutrophilic infiltration are still not known. It may be a reason for parenchymal damage and vascular injury[18]. In the current study, the prophylactic administration of G-CSF supported the maintenance of epithelial structure against ionising radiation, suggesting that further stimulation of tissue neutrophil accumulation by G-CSF in the intestine may protect against radiation-induced mucosal injury. It appears likely that G-CSF inhibits the translocation of bacterial flora, which may become pathogenic as the mucosal barrier is broken down by irradiation. Furthermore, neutrophil influx established by G-CSF may also be effective against ROS-induced mucosal damage due to irradiation.

The results of the present study confirm that G-CSF maintains the intestinal mucosal structure when given as a pre-treatment before irradiation of the pelvic area. G-CSF stimulates the production of white blood cells and it is used in certain cancer patients to accelerate recovery from neutropenia after chemotherapy, allowing higher-intensity treatment regimens. Thus, it may be also relevant to expect G-CSF to support the radiotherapy as an adjuvant therapeutic. Nevertheless, further studies are required to investigate the exact mechanisms responsible for the protective effects of G-CSF.

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