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Effect of granulocyte-macrophage colony-stimulating factor on treatment of acute osteomyelitis

An experimental investigation in rats

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Abstract Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that affects the various developmental steps of hematopoietic cells and enhances the phagocytic activity of these cells. The effect of GM-CSF on acute osteomyelitis, developed in rats, was investigated. For this purpose, osteomyelitis was firstly developed through the direct inoculation of *Staphylococcus aureus* into rat tibial metaphysis. Twenty-four rats in which diagnosis of osteomyelitis was histopathologically established were divided into two groups. Antibiotic only was given to the first group, and antibiotic as well as GM-CSF to the second group. Rats were followed up for 3 months with plain radiographs and scintigraphic methods using ^{67}Ga-citrate. Material obtained from the rats that had been killed at the end of the 3rd month were histopathologically investigated. One rat in the first group died. In another rat, chronic osteomyelitis developed and fracture was observed. In 12 rats of the second group, physical examination, plain radiographs, and histopathologic findings were normal. In scintigraphic studies with ^{67}Ga-citrate, when the pre- and posttreatment values of the same groups were evaluated by the Mann-Whitney *U*-test, the mean values at 48 h after treatment were found to be significant ($P < 0.05$), indicating a decrease in the 2nd group (experimental group). In conclusion, the antibiotics were effective in the elimination of infection only together with neutrophils. In this manner, infections may be eliminated by strengthening the host's defense mechanism as well as by administering antibiotics. We believe that an adequate number of long-term studies will shed light on this issue. Besides we consider that this factor will be more important in the study of chronic osteomyelitis.

Keywords Granulocyte-macrophage colony-stimulating factor · Osteomyelitis

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Introduction

Acute osteomyelitis is treated currently by the drainage of purulent material and administration of antibiotics for an appropriate period and at an appropriate dose, alongside symptomatic treatment. Since this treatment protocol cannot prevent chronicity in some acute osteomyelitis patients, discussions on the period for administering the antibiotics [3] and the timing of surgical drainage are still going on [5, 10]. The strengthening of the host's defense mechanism plays an important role in removing the infection. Thus, the number of studies on the host's defense mechanism have increased, owing to the belief that, alongside the externally administered treatment, regional and systemic defense mechanisms should be particularly active.

The information on hematopoietic growth factors (HGFs) that are influential in the defense mechanism of the host was discovered for the first time in 1977 [2]. In the following years, comprehensive information on molecular and cellular biology of HGFs was gathered [6]. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a HGF that stimulates the proliferation of cells such as neutrophils, monocytes, and eosinophils and that increases the phagocytotic and killer capabilities of such cells. Owing to such characteristics, in recent times GM-CSF has been used for the treatment of infectious diseases as well as in cancer treatment [1].

This study aims at investigating the efficiency of GM-CSF in the treatment of osteomyelitis in rats, in which osteomyelitis has been induced, by taking into account the fact that GM-CSF increases the functions of mature leukocytes, as well as its other functions.

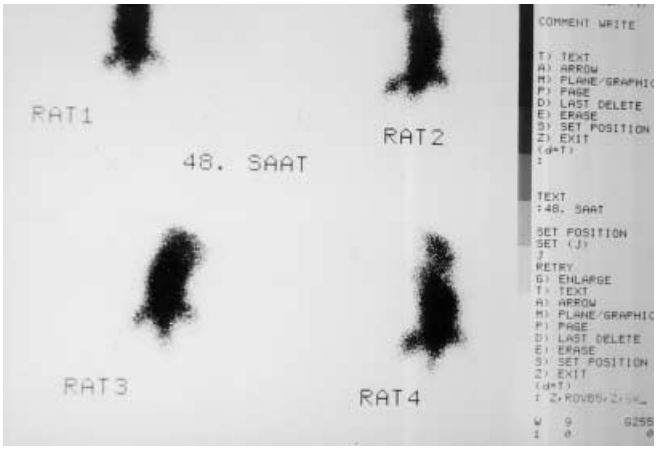

Fig. 1 ^{67}Ga-citrate whole-body bone scintigraphy of rat

Materials and methods

This study was approved by the University of Dicle ethics committee for animal experimentation and conforms to the National Institutes of Health guidelines for the care and use of laboratory animals.

Sprague Dawley rats of 1.5 months produced in the Animal Research Laboratory of Dicle University and weighing between 150 and 200 g were used as study subjects. The pathogen *Staphylococcus aureus*, which was prepared according to the turbidity of a McFarland 0.5th tube in the microbiology laboratory, was used to create the infection. The rats were anesthetized with ketamine hydrochloride (0.8 mg/kg) intramuscularly, and the proximal extremity of the tibia was prepared for operation under sterile conditions. A 1-cm skin incision was made in order to reach the bone. The cortex was opened by a Kirshner wire, with a fine point and a diameter of 0.5 mm, and the medulla was then reached. Then, 0.05 ml (approximately 10^4) living microorganisms were inoculated into the medullar region and the opening was closed with dental cement. The skin was sutured at the end of the operation.

Seventy-two hours after the inoculation of microorganism into the metaphyseal region, quantitative static measurements of all the rats were realised histopathologically and by scintigraphy at 24 h, 48 h, and 72 h with ^{67}Ga-citrate (Fig. 1). As a result of the scintigraphic investigation with ^{67}Ga-citrate, acute osteomyelitis was observed in 32 animals. Twenty-four of these rats were divided into two groups and different treatments were applied to each group.

Twelve rats in the 1st group were administered ampicillin-sulbactam (Combisid) parenterally for 3 weeks at a dosage of 150 mg/kg per day in two equal doses.

Twelve rats in the 2nd group were administered GM-CSF intramuscularly for 7 days at a dosage of 5 μg/kg per day in addition to the antibiotics administered at the same dosage in the last group.

Results

The rats under treatment were observed by daily physical examination and radiographics taken with 3-week intervals. The microorganism were evaluated 72 h after inoculation into the metaphyseal region and by scintigraphy, which was repeated at the end of the 3rd month. Furthermore, they were examined histopathologically at day 7 and at the end of the 3rd month.

Group 1

One of the rats died during scintigraphic investigations. As a result of the investigations made at the end of the 3rd month, chronic infection occurred in 1 of the remaining 11 rats and later on a pathological fracture occurred. The physical examination and radiographics of another 10 rats were evaluated without incident.

Group 2

The physical examination and radiographics of all the subjects investigated were evaluated as usual. At the end of the 3rd month, the rats were administered 1 mCi ^{67}Ga-citrate and the static images at the 24 h, 48 h, and 72 h were repeated (Figs. 2, 3). The region of interest (ROI) in the left leg in which osteomyelitis was induced and in the same region of the right leg were drawn and counts were obtained and analyzed. When the pre- and posttreatment mean

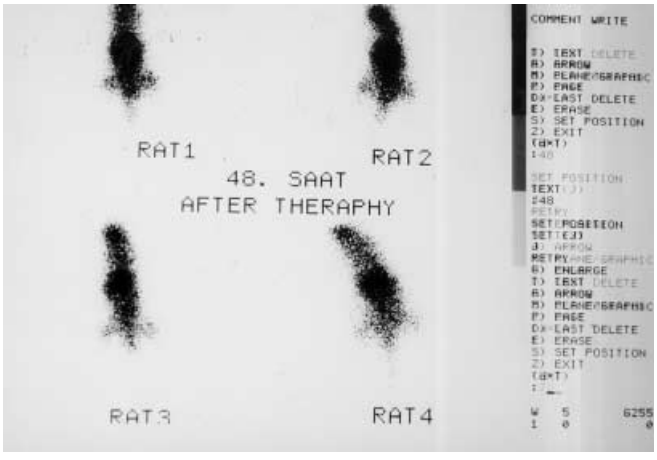

Fig. 2 Whole-body bone ^{67}Ga-citrate scintigraphy in granulocyte-macrophage colony-stimulating factor (GM-CSF)-administered rat group

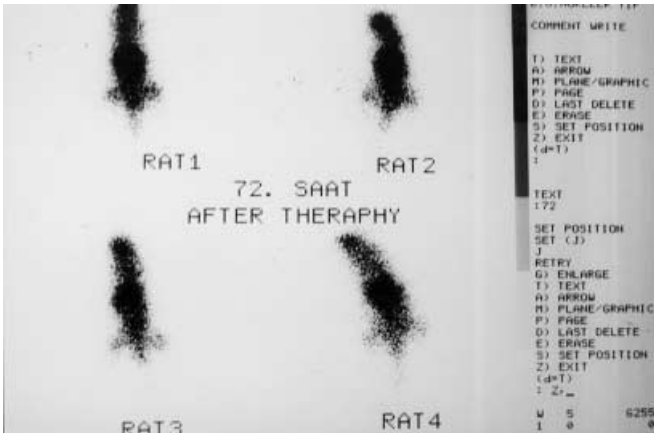

Fig. 3 After antibiotic administration, whole-body bone ^{67}Ga-citrate scintigraphy of rat

involvement values of both groups were evaluated by the Wilcoxon test within themselves, the decrease in the involvement (activity) was found significant ($P < 0.01$) and when the pre- and posttreatment values of same groups were evaluated by the Mann-Whitney *U*-test, the mean values at 48 h after treatment were found significant ($P < 0.05$), showing a decrease in the 2nd group (experimental group).

Biopsies were taken from all rats on day 7 (on which GM-CSF administration was terminated). At the end of the histopathological analysis, a significant increase in leukocyte numbers was observed in group 2, whereas there was less of an increase in group 1. Furthermore, in the histopathological examinations made at the end of the 3rd month, it was observed that the findings were similar in the rats, except for one, which developed chronic osteomyelitis, and that there was intensive chronic infectious cell infiltration in the form of focal areas at some parts (Fig. 4). In the second group, although chronic infectious cell infiltration was observed, the infiltration was milder (Fig. 5).

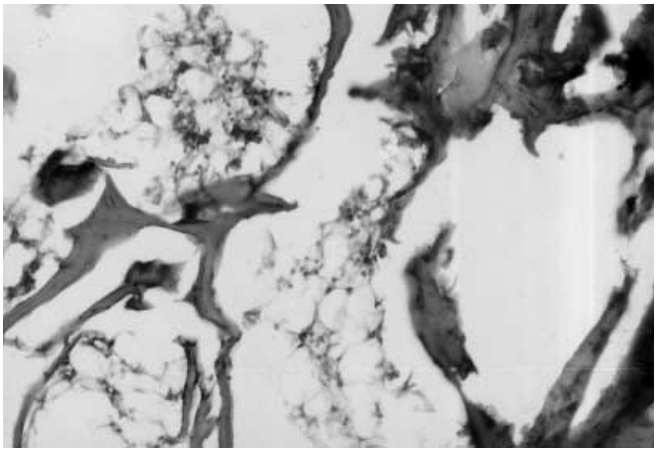

Fig. 4 Significant lymphocytic infiltration between bone trabecules (H.E.; × 100)

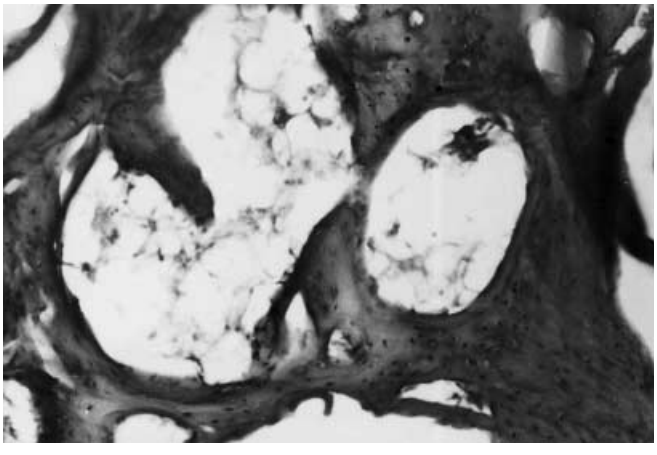

Fig. 5 Rare lymphocytic infiltration between bone trabecules (H.E.; × 100)

Discussion

In recent years, some difficulties have been observed in the treatment of infections due to the fact that microorganisms use immune mechanisms against antimicrobial agents. The wrong and excessive use of antibiotics has led to nearly all pathogenic microorganisms developing immunity against antibiotics. Although infections were treated successfully by antibiotics alone in the years when antibiotics were first introduced, the same rate of success has not been achieved in recent years by the use of various treatment protocols [5]. The surgical drainage that was practiced in the preantibiotic period is being considered again. Some authors have claimed that the addition of surgical drainage would increase the chance of success [10], whereas some other authors claim that surgery has no place in the acute phase of a disease [9]. In spite of all these discussions, the change from acute to chronic osteomyelitis cannot be prevented in some cases [5, 7].

Recently, studies on changing the response of the host toward infection have been interesting. Another reason for research in this field is that existing antibiotics may effect microorganisms other than those targeted. In light of these arguments, recently some HGFs that can stimulate various myeloid cell groups have attracted great attention owing to the capability of the phagocytes to increase their numbers and functions in the course of infection. HGFs increase the number of hematopoietic cells related to the defense of the host, as well as increasing the chemotaxis of these cells and extending their life cycle. Furthermore, they increase the phagocytic activity of the hematopoietic cells [8]. Because of such an effect, various studies have been conducted on the view that they may be useful in host defense against infections both in neutropenic and nonneutropenic hosts [4].

GM-CSF is one of the hematopoietic factors that plays a role in the early primary stage of the marrow and that affects the formation of neutrophil, eosinophil, and monocyte. By the role it has played in various stages of the host's defense mechanism, GM-CSF has been proved to be a major factor in the increase in phagocytosis. In light of these findings, we tested the effect of GM-CSF on animals suffering from osteomyelitis. In the scintigraphic study, pretreatment and posttreatment mean values of group 1 at 24 h, 48 h, and 72 h, and the mean values of group 2 were proved significantly different ($P < 0.01$) when evaluated with the Wilcoxon test. Using ^{67}Ga-citrate, pretreatment and posttreatment quantitative scintigraphic values of both groups were compared. Forty-eight hours after the treatment, a significant difference ($P < 0.05$) was observed according to the Mann-Whitney *U*-test. While there was very little involvement in the first 24 h, it increased up to the highest level at 48 h and decreased at 72 h.

During the pathological observations that were carried out in week 1, an outstanding increase in leukocytes was observed. No such increase was observed in group 1. The increase conforms with scintigraphic findings. The in-

crease in leukocytes may also be dependent on the regular defense mechanism of the body. In order to make a distinction, what is required is a study of the functions of the leukocytes (which could not be realized owing to practicalities). The histopathological observations that were carried out at the end of the 3rd month have proved our views correct.

In conclusion, the administered antibiotics were effective in the elimination of infection only together with neutrophils. In this manner, infections may be eliminated by strengthening the host's defense mechanism as well as using antibiotics. In light of this view, we consider that success rate will increase by including GM-CSF into the treatment. The number of incidents and the failure to carry out controls over a long period of time prevented us from achieving a conclusive result. We believe that adequate numbers of long-term studies will shed light on this issue. Also we consider that GM-CSF will be most useful in the study of chronic osteomyelitis.

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