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The effect of $FeCl_3$ on the accumulation of gallium-67 into inflammatory and normal tissues

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The effect of $FeCl_3$ on the uptake of ^{67}Ga by inflammatory and normal tissues was studied to clarify the role of transferrin in ^{67}Ga uptake by inflammatory tissue. The administration of $FeCl_3$ 5 min before the injection of ^{67}Ga decreased the uptake of ^{67}Ga by liver and spleen, but had little effect on the uptake of ^{67}Ga by the inflammatory tissue. These results suggest that ^{67}Ga is taken up by normal tissues in a transferrin-bound form but in an unbound form by inflammatory tissue. On the other hand, when $FeCl_3$ was simultaneously injected with ^{67}Ga, the uptake of ^{67}Ga by liver and spleen was markedly increased but the uptake by inflammatory tissue was decreased.

Key words: ^{67}Ga uptake, Inflammation, $FeCl_3$

INTRODUCTION

GALLIUM-67 has been used for the detection of various tumors[1] and acute and chronic inflammation.[2,3] Many hypotheses concerning the mechanism of the uptake of ^{67}Ga by tumors and inflammatory lesions[4–5] have been proposed. Tsan et al.[8] and Vallabhajosula et al.[9] reported that ^{67}Ga was almost all bound to transferrin, iron-binding protein, in blood. It was reported that transferrin played a major role for the mechanism of the uptake of ^{67}Ga by tumors.[10–13] On the other hand, it was shown that transferrin transports ^{67}Ga to the tumor tissue but that ^{67}Ga is taken up by tumor cells in an unbound form.[7,14–16] The involvement of transferrin in the uptake of ^{67}Ga by inflammatory tissue has not, however, been conclusively demonstrated. We have recently demonstrated that ^{67}Ga densely accumulates in granuloma tissue, that is inflammatory tissue produced by turpentine oil.[17] In the present study using $FeCl_3$, we have investigated whether or not transferrin is involved in the uptake of ^{67}Ga by the inflammatory tissue.

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MATERIALS AND METHODS

Animals

Male Wistar rats weighing 150–200 g were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan), and were housed in wire mesh cages at a room temperature of $23\pm1°C$ and in relative humidity of $55\pm5\%$.

Production of inflammatory tissue

The production of inflammatory tissue, granuloma, was carried out by the same method described in the previous report[17] as follows. A paper pellet (size 8 mm) was dipped in turpentine oil and implanted bilaterally in the subcutaneous tissues of the abdomen in each animal.

^{67}Ga solution

Gallium-67 citrate (kindly supplied by Nihon Mediphysics Co., Ltd., Takarazuka, Japan) was diluted with saline to 185 kBq (5 μCi)/m*l*.

Administration of ^{67}Ga

Each rat, at 6 days after the administration of turpentine oil, was intravenously injected with ^{67}Ga in a dose of 37 kBq (200 μl).

Administration of $FeCl_3$

Each rat, at 6 days after the administration of turpentine oil, was intravenously injected with $FeCl_3$ (6.25–25 μmole/m*l* saline) in a dose of 100 μl 5 min before or 30 min after the administration of ^{67}Ga.

The simultaneous administration of $FeCl_3$ and ^{67}Ga was carried out by the intravenous injection of $FeCl_3$ saline solution (12.5 μmole/m*l*) containing ^{67}Ga (185 kBq) in a dose of 200 μl.

Removal of granuloma and other tissues

At 4 hrs after the administration of ^{67}Ga, rats were anesthetized with urethane (1.5 g/kg, i.p.) and immediately perfused with cold saline. The inflammatory lesion and other tissues were then removed. The granuloma tissue was obtained from the inflammatory lesion with complete removal of the implanted paper pellet and abscess.

Determination of radioactivity

Radioactivity of various tissues removed was determined with a well-type NaI-scintillation counter (Aloka, ARC-300). The uptake ratios of ^{67}Ga in various tissues were expressed in the following formula: Uptake ratio=sample radioactivity (cpm)/sample weight (g)/total radioactivity administered (cpm)/body weight of rat (g).

RESULTS

The effect of various amounts of $FeCl_3$ *on the uptake of* ^{67}Ga *by inflammatory and normal tissues*

To investigate the effect of $FeCl_3$ on the retention of ^{67}Ga in the blood and ^{67}Ga uptake by inflammatory and normal tissues, *in vivo* experiments were performed (Fig. 1). $FeCl_3$ (0.625, 1.25, and 2.5 μmole/rat) was administered 5 min before the injection of ^{67}Ga and the blood, urine, inflammatory and other tissues were removed 4 hrs later. $FeCl_3$ dose dependently reduced ^{67}Ga retention in the blood. Similarly the uptake of ^{67}Ga by the liver and spleen was dose dependently reduced. In contrast to this, $FeCl_3$ (2.5 μmole/rat) markedly increased the excretion of ^{67}Ga into the urine. On the other hand, $FeCl_3$ had little effect on the uptake of ^{67}Ga into the inflammatory tissue, granuloma.

The effect of the time of $FeCl_3$ *administration on the uptake of* ^{67}Ga *in inflammatory and normal tissues*

The administration of $FeCl_3$ (2.5 μmole/rat) 30 min after the injection of ^{67}Ga decreased the uptake ratios of ^{67}Ga into the blood more than that 5 min before (Fig. 2). On the other hand, the administration of $FeCl_3$ 30 min after the injection of ^{67}Ga increased the uptake of ^{67}Ga by the liver and spleen more than 5 min before. But in all cases, the blood, liver and spleen uptake ratios of ^{67}Ga were greatly decreased by the administration of $FeCl_3$ as compared with ^{67}Ga alone. On the other hand, concerning the uptake ratio of ^{67}Ga into granuloma, there is no difference between the administration of $FeCl_3$ 5 min before and 30 min after the injection of ^{67}Ga, although the uptake ratios in both cases were slightly decreased by the administration of $FeCl_3$.

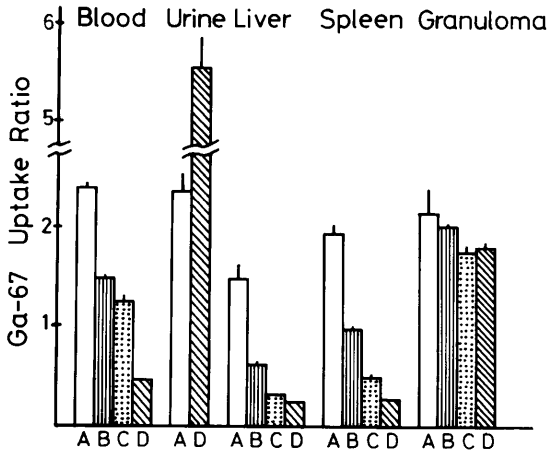

Fig. 1 Effect of various amounts of $FeCl_3$ on the uptake of ^{67}Ga by several tissues. A: ^{67}Ga alone. B: 0.625, C: 1.25 and D: 2.5 μmole/rat of $FeCl_3$ were administered 5 min before the injection of ^{67}Ga.
Each point represents the mean and SEM for five rats.

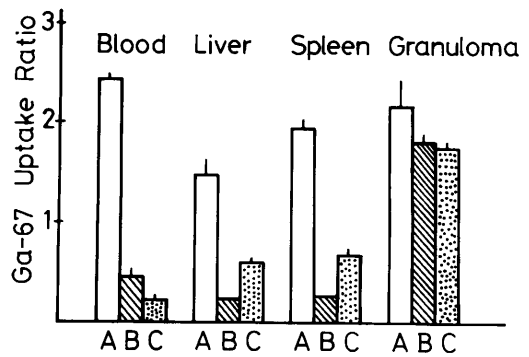

Fig. 2 Effect of the time of $FeCl_3$ administration on the uptake of ^{67}Ga by several tissues. A: ^{67}Ga alone. $FeCl_3$ (2.5 μmole/rat) was administered 5 min before (B) and 30 min after (C) the injection of ^{67}Ga.
Each point represents the mean and SEM for five rats.

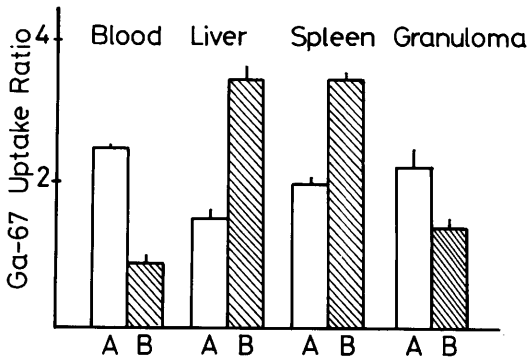

Fig. 3 Effect of the simultaneous administration of $FeCl_3$ on the uptake ratio of ^{67}Ga by several tissues. A: ^{67}Ga alone. B: ^{67}Ga was injected along with $FeCl_3$.
Each point represents the mean and SEM for five rats.

The effect of the simultaneous administration of $FeCl_3$ *and* ^{67}Ga *on the uptake ratio of* ^{67}Ga *into inflammatory and normal tissues*

The simultaneous administration of $FeCl_3$ and ^{67}Ga greatly increased the uptake ratios of ^{67}Ga into liver and spleen as compared with ^{67}Ga alone but decreased those into blood and granuloma (Fig. 3). The decrease in the uptake ratio of ^{67}Ga into blood by the simultaneous administration was, however, much smaller than that following the administration of $FeCl_3$ 5 min before and 30 min after the injection of ^{67}Ga.

DISCUSSION

We used ferric chloride as the iron reagent because ferric citrate poorly disolved in saline, although Hayes et al.[6] used ferric citrate to investigate the effect of iron on the distribution of ^{67}Ga. They have reported that ferric citrate slightly increased the blood distribution of ^{67}Ga. The results from Fig. 1 in the present study indicate, however, that $FeCl_3$ inhibits the binding of ^{67}Ga to transferrin, iron binding protein, in blood, so that the retention of ^{67}Ga in blood is greatly reduced by $FeCl_3$ and ^{67}Ga in an unbound form due to $FeCl_3$ is excreted in urine. Additionally $FeCl_3$ can decrease the uptake of ^{67}Ga by liver and spleen without disturbing the uptake of ^{67}Ga by the inflammatory lesions.

Hayes et al.[6] reported that the uptake of ^{67}Ga by normal tissues such as liver or spleen occurred to a major extent by endocytosis in which transferrin is involved, and that the initial entry of ^{67}Ga into inflammatory lesions might occur in the same way as to that of normal soft tissues, while the uptake of ^{67}Ga by tumors occurs mainly by the diffusion of the unbound form. Vallabhajosula et al.[7] reported that the uptake of ^{67}Ga into tumors was reduced by transferrin, as compared with ^{67}Ga-citrate alone, i.e., the uptake of ^{67}Ga by tumors occurred in an unbound form. In the present study, the results indicating that transferrin is involved in the uptake of ^{67}Ga by liver and spleen are consistent with those obtained by Hayes et al.,[6] although the effect of the ferric citrate used by them on the liver and spleen was weaker than that of the ferric chloride used by us. We think, however, that the uptake of ^{67}Ga into inflammatory tissue occurs in an unbound form in the same way as into tumors. It should be noted, however, that the inflammatory lesions employed by Hayes et al. were abscesses but those employed by us were granuloma tissues, not containing an abscess. Oberhaensli et al.[19] have also reported that iron decreased the distribution of ^{67}Ga int the liver and abscess. Both they and Hayes et al.[6] injected iron simultaneously with ^{67}Ga. Their results are quite the opposite of ours. In our results the simultaneous injection of iron increased the uptake of ^{67}Ga into liver and spleen. We cannot understand these differences. They must inject iron and ^{67}Ga by separate routes. Moreover, Hayes et al.[6] have reported that iron increased the distribution of ^{67}Ga in blood, although that in liver was decreased with iron. These results are unreasonable. The results from the present study show that iron can decrease the amount of ^{67}Ga in blood but cannot remove the ^{67}Ga that has already entered the liver and spleen. Since almost all ^{67}Ga is, however, bound to transferrin in blood,[8,9] the decrease in ^{67}Ga in blood due to $FeCl_3$ is the decrease in ^{67}Ga in a bound form. Therefore the decrease in the uptake of ^{67}Ga into liver or spleen when $FeCl_3$ was administered resulted from the decrease in ^{67}Ga binding to transferrin. On the other hand, $FeCl_3$ had little effect on the uptake of ^{67}Ga by granuloma, i.e., ^{67}Ga might accumulate in the inflammatory tissue either in a transferrin bound form or in an unbound form.

Many workers have expressed confidence that ^{67}Ga is exclusively bound to and transported to various tissues by transferrin in the blood. At the tumor or inflammatory site, ^{67}Ga is then dissociated from the transferrin complex. Vallabhajosula et al.[16,18] reported that acidic pH at the tumor site might be one of the factors involved in ^{67}Ga localization in tumors. Moreover, reduction of the pH to 6.8–7.0, below that of normal tissue (7.4), might be due to the accumulation of lactic acid produced by anaerobic glycolysis. We think that the pH at the granuloma site must also be reduced by anaerobic glycolysis. We think that ^{67}Ga is transported to the inflammatory site either in a transferrin bound form or in an unbound form. Gallium-67 in an unbound form directly enters inflammatory tissue, whereas, ^{67}Ga in a bound form is dissociated under acidic conditions at the inflammatory site and then enters the inflammatory tissue. We conclude that transferrin is not involved in the uptake of ^{67}Ga into inflammatory tissue, such as granuloma, and that the uptake of ^{67}Ga uptake into inflammatory tissue may be similar to that occurring in tumor tissues.

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