The effect of FeCl₃ on the accumulation of gallium-67 into inflammatory and normal tissues

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The effect of FeCl₃ on the uptake of ⁶⁷Ga by inflammatory and normal tissues was studied to clarify the role of transferrin in ⁶⁷Ga uptake by inflammatory tissue. The administration of FeCl₃ 5 min before the injection of ⁶⁷Ga decreased the uptake of ⁶⁷Ga by liver and spleen, but had little effect on the uptake of ⁶⁷Ga by the inflammatory tissue. These results suggest that ⁶⁷Ga is taken up by normal tissues in a transferrin-bound form but in an unbound form by inflammatory tissue. On the other hand, when FeCl₃ was simultaneously injected with ⁶⁷Ga, the uptake of ⁶⁷Ga by liver and spleen was markedly increased but the uptake by inflammatory tissue was decreased.

Key words: ⁶⁷Ga uptake, Inflammation, FeCl₃

INTRODUCTION

Gallium-67 has been used for the detection of various tumors¹ and acute and chronic inflammation.²-⁸ Many hypotheses concerning the mechanism of the uptake of ⁶⁷Ga by tumors and inflammatory lesions⁴-⁵ have been proposed. Tsan et al.⁹ and Vallabhajosula et al.⁹ reported that ⁶⁷Ga 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 ⁶⁷Ga by tumors.¹⁰-¹³ On the other hand, it was shown that transferrin transports ⁶⁷Ga to the tumor tissue but that ⁶⁷Ga is taken up by tumor cells in an unbound form.²,¹⁴-¹⁶ The involvement of transferrin in the uptake of ⁶⁷Ga by inflammatory tissue has not, however, been conclusively demonstrated. We have recently demonstrated that ⁶⁷Ga densely accumulates in granuloma tissue, that is inflammatory tissue produced by turpentine oil.¹⁷ In the present study using FeCl₃, we have investigated whether or not transferrin is involved in the uptake of ⁶⁷Ga by the inflammatory tissue.

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The simultaneous administration of FeCl₃ and ⁶⁷Ga was carried out by the intravenous injection of FeCl₃ saline solution (12.5 μmole/ml) containing ⁶⁷Ga (185 kBq) in a dose of 200 μl.

Removal of granuloma and other tissues
At 4 hrs after the administration of ⁶⁷Ga, 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 ⁶⁷Ga 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 FeCl₃ on the uptake of ⁶⁷Ga by inflammatory and normal tissues
To investigate the effect of FeCl₃ on the retention of ⁶⁷Ga in the blood and ⁶⁷Ga uptake by inflammatory and normal tissues, in vivo experiments were performed (Fig. 1). FeCl₃ (0.625, 1.25, and 2.5 μmole/rat) was administered 5 min before the injection of ⁶⁷Ga and the blood, urine, inflammatory and other tissues were removed 4 hrs later. FeCl₃ dose dependently reduced ⁶⁷Ga retention in the blood. Similarly the uptake of ⁶⁷Ga by the liver and spleen was dose dependently reduced. In contrast to this, FeCl₃ (2.5 μmole/rat) markedly increased the excretion of ⁶⁷Ga into the urine. On the other hand, FeCl₃ had little effect on the uptake of ⁶⁷Ga into the inflammatory tissue, granuloma.

The effect of the time of FeCl₃ administration on the uptake of ⁶⁷Ga in inflammatory and normal tissues
The administration of FeCl₃ (2.5 μmole/rat) 30 min after the injection of ⁶⁷Ga decreased the uptake ratios of ⁶⁷Ga into the blood more than that 5 min before (Fig. 2). On the other hand, the administration of FeCl₃ 30 min after the injection of ⁶⁷Ga increased the uptake of ⁶⁷Ga by the liver and spleen more than 5 min before. But in all cases, the blood, liver and spleen uptake ratios of ⁶⁷Ga were greatly decreased by the administration of FeCl₃ as compared with ⁶⁷Ga alone. On the other hand, concerning the uptake ratio of ⁶⁷Ga into granuloma, there is no difference between the administration of FeCl₃ 5 min before and 30 min after the injection of ⁶⁷Ga, although the uptake ratios in both cases were slightly decreased by the administration of FeCl₃.

![Fig. 1 Effect of various amounts of FeCl₃ on the uptake of ⁶⁷Ga by several tissues. A: ⁶⁷Ga alone. B: 0.625, C: 1.25 and D: 2.5 μmole/rat of FeCl₃ were administered 5 min before the injection of ⁶⁷Ga. Each point represents the mean and SEM for five rats.](image1)

![Fig. 2 Effect of the time of FeCl₃ administration on the uptake of ⁶⁷Ga by several tissues. A: ⁶⁷Ga alone. FeCl₃ (2.5 μmole/rat) was administered 5 min before (B) and 30 min after (C) the injection of ⁶⁷Ga. Each point represents the mean and SEM for five rats.](image2)

![Fig. 3 Effect of the simultaneous administration of FeCl₃ on the uptake ratio of ⁶⁷Ga by several tissues. A: ⁶⁷Ga alone. B: ⁶⁷Ga was injected along with FeCl₃. Each point represents the mean and SEM for five rats.](image3)
The effect of the simultaneous administration of FeCl₃ and ⁶⁷Ga on the uptake ratio of ⁶⁷Ga into inflammatory and normal tissues

The simultaneous administration of FeCl₃ and ⁶⁷Ga greatly increased the uptake ratios of ⁶⁷Ga into liver and spleen as compared with ⁶⁷Ga alone but decreased those into blood and granuloma (Fig. 3). The decrease in the uptake ratio of ⁶⁷Ga into blood by the simultaneous administration was, however, much smaller than that following the administration of FeCl₃ 5 min before and 30 min after the injection of ⁶⁷Ga.

DISCUSSION

We used ferric citrate as the iron reagent because ferric citrate poorly dissolved in saline, although Hayes et al.⁶ used ferric citrate to investigate the effect of iron on the distribution of ⁶⁷Ga. They have reported that ferric citrate slightly increased the blood distribution of ⁶⁷Ga. The results from Fig. 1 in the present study indicate, however, that FeCl₃ inhibits the binding of ⁶⁷Ga to transferrin, iron binding protein, in blood, so that the retention of ⁶⁷Ga in blood is greatly reduced by FeCl₃ and ⁶⁷Ga in an unbound form due to FeCl₃ is excreted in urine. Additionally FeCl₃ can decrease the uptake of ⁶⁷Ga by liver and spleen without disturbing the uptake of ⁶⁷Ga by the inflammatory lesions.

Hayes et al.⁶ reported that the uptake of ⁶⁷Ga 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 ⁶⁷Ga into inflammatory lesions might occur in the same way as to that of normal soft tissues, while the uptake of ⁶⁷Ga by tumors occurs mainly by the diffusion of the unbound form. Vallabhajosula et al.⁷ reported that the uptake of ⁶⁷Ga into tumors was reduced by transferrin, as compared with ⁶⁷Ga-citrate alone, i.e., the uptake of ⁶⁷Ga by tumors occurred in an unbound form. In the present study, the results indicating that transferrin is involved in the uptake of ⁶⁷Ga 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 ⁶⁷Ga 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.⁹ have also reported that iron decreased the distribution of ⁶⁷Ga into the liver and abscesses. Both they and Hayes et al.⁶ injected iron simultaneously with ⁶⁷Ga. Their results are quite the opposite of ours. In our results the simultaneous injection of iron increased the uptake of ⁶⁷Ga into liver and spleen. We cannot understand these differences. They must inject iron and ⁶⁷Ga by separate routes. Moreover, Hayes et al.⁶ have reported that iron increased the distribution of ⁶⁷Ga 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 ⁶⁷Ga in blood but cannot remove the ⁶⁷Ga that has already entered the liver and spleen. Since almost all ⁶⁷Ga is, however, bound to transferrin in blood,⁸,⁹ the decrease in ⁶⁷Ga in blood due to FeCl₃ is the decrease in ⁶⁷Ga in a bound form. Therefore the decrease in the uptake of ⁶⁷Ga into liver or spleen when FeCl₃ was administered resulted from the decrease in ⁶⁷Ga binding to transferrin. On the other hand, FeCl₃ had little effect on the uptake of ⁶⁷Ga by granuloma, i.e., ⁶⁷Ga might accumulate in the inflammatory tissue either in a transferrin bound form or in an unbound form.

Many workers have expressed confidence that ⁶⁷Ga is exclusively bound to and transported to various tissues by transferrin in the blood. At the tumor or inflammatory site, ⁶⁷Ga 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 ⁶⁷Ga 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 ⁶⁷Ga 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 ⁶⁷Ga 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 ⁶⁷Ga into inflammatory tissue, such as granuloma, and that the uptake of ⁶⁷Ga uptake into inflammatory tissue may be similar to that occurring in tumor tissues.

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