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# Effect of edetate calcium disodium on yttrium-90 activity in bone of mice

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The kinetics of Yttrium-90 (Y-90) in bone of mice was investigated in combination with edetate calcium disodium ($CaNa_2EDTA$). One group of mice were intraperitoneally administered 37.5 mg/kg $CaNa_2EDTA$ or 0.9% NaCl as a control at 1, 22, 34, 46, 58, 70, 82, 94, 154 and 166 h after injection of Y-90 acetate (post-administration), and the biodistribution was studied at 3, 24, 72, 120 and 168 h postinjection of Y-90 acetate. No difference between the post-$CaNa_2EDTA$-treated mice and the control was demonstrated in the radioactivity in the bone. A decrease in radioactivity in the liver and kidneys was accelerated, and the radioactivity was lower than the control at 120 h postinjection. The other group of mice were also given the same dose of chelator at 12 h and 1 h preinjection of Y-90 acetate and at 1, 22, 34, 46, 58, 70, 82, 94, 154 and 166 h after injection of Y-90 acetate (pre- and post-administration), the radioactivity in bone at 3 h postinjection was significantly lower than in the control ($24.4 \pm 3.92\%$ ID/g vs. $31.7 \pm 2.26\%$ ID/g, $p < 0.05$), but the decrease was not sequential. A significant reduction in radioactivity in the blood, kidneys and liver was demonstrated at 3 h, 72 h and 72 h postinjection. In conclusion, the $CaNa_2EDTA$ with the administration schedule employed here cannot chelate the Y-90 from bone but the free Y-90 before deposition into bone.

**Key words:** edetate calcium disodium, bone uptake

## INTRODUCTION

YTTRIUM-90 (Y-90) is a candidate radionuclide for radioimmunotherapy because of its high maximum energy of 2.3 MeV, long-range beta-emitting character (maximum tissue penetration of nearly 1 cm with 80% of the energy deposited in the first 4 to 5 mm), and half-life of 64.1 h but Y-90 dissociated from Y-90-labeled-monoclonal antibody (MAb) specifically accumulates in bone.[1–6] The bone uptake of Y-90 may cause myelosuppression, and prevent its therapeutic potential from being realized. The use of a therapeutic chelating agent for heavy metal poisoning, edetate calcium disodium ($CaNa_2EDTA$), to suppress the undesirable radioactivity in bone, was investigated.[7] The administration of $CaNa_2EDTA$ reduced the radioactivity in bone and safely allowed the dose of Y-90-labeled-MAb to be increased to a sufficient level for therapy without severe myelosuppression.[8,9] We have also confirmed the effect of $CaNa_2EDTA$ on the radioactivity in the bone of tumor-bearing nude mice with Y-90 labeled MAb[10] but it was not clear whether the reduced radioactivity in the bone resulted from chelating of Y-90 from bone or from removal of free Y-90 that did not localize in bone. In this study, the kinetics of Y-90-activity in bone of mice was investigated in combination with $CaNa_2EDTA$, and the mechanism of $CaNa_2EDTA$-mediated reduction in radioactivity in bone is discussed.

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## MATERIALS AND METHODS

*Animals*

Female normal mice with a Balb/c background were

obtained from the Institute of Experimental Animal Research, Gunma University School of Medicine at 4 weeks of age (16.5 g–18.7 g mouse body weight). The mice were allowed free access to commercial chow and distilled-deionized water during the experiment. The ambient temperature during the study was maintained between 21°C and 23°C, and the mice were exposed to a 12-h light, 12-h dark cycle. Animal studies were performed in compliance with relevant regulations relating to the conduct of animal experiments issued by Gunma University.

*Effect of $CaNa_2EDTA$ on biodistribution in normal mice injected with Y-90 acetate*

One group of mice were slowly, intraperitoneally administered 37.5 mg/kg $CaNa_2EDTA$ solution (Bleian Inj.®, Nisshin Pharmacy Co. Ltd., Yamagata, Japan) or an equal volume of 0.9% NaCl solution as a control at 1, 22, 34, 46, 58, 70, 82, 94, 154 and 166 h after intravenous injection of Y-90 acetate (740 kBq) (post-administration). The Y-90 acetate (925 MBq/mL) was prepared by mixing Y-90-chloride in 50 mM HCl (1850 MBq/mL) (Nordion, Kanata, Ontario, Canada) with an equal volume of 1 M sodium acetate buffer, pH 5.8, for 30 min at room temperature. The other group of mice were slowly intraperitoneally administered 37.5 mg/kg $CaNa_2EDTA$ solution or an equal volume of 0.9% NaCl solution as a control at 12 h and 1 h before and at 1, 22, 34, 46, 58, 70, 82, 94, 154 and 166 h after intravenous injection of Y-90 acetate (740 kBq) (pre- and post-administration). Every five mice were sacrificed by heart puncture under general anesthesia at 3, 24, 72, 120 and 168 h postinjection of Y-90 acetate. Circulating blood was then replaced by 0.9% NaCl with 0.01% heparin: the right atrium was cut while 0.9% NaCl with 0.01% heparin was mildly perfused into the left ventricle with a small syringe. The liver, kidneys, stomach, intestines, spleen, lungs, muscle (quadriceps) and bone (femur) were removed and cut into pieces weighing less than 200 mg. The radioactivity of each samples was counted in a NaI well-type gamma counter (Aloka, Tokyo, Japan) with an energy window ranging from 100 keV to ∞. The counting efficiency of the NaI well-type gamma counter in measuring Y-90 preparation was approximately 12%. A sufficient linearity between radioactivity and count rate was obtained when non-dissolved samples were below 200 mg in weight. Results were expressed as a percent injected dose/gram tissue (% ID/g) normalized to 20 g mouse. Serum calcium levels from blood of mice pre- and post-administered $CaNa_2EDTA$ and 0.9% NaCl were measured at 168 h postinjection by the *ortho*-cresolphtalein complexone (OCPC) method to check for possible side effects of the chelator.

*Data analysis*

Statistical comparison of values was made by Student's unpaired t-test. Differences were considered statistically significant when a $p$ value was less than 0.05.

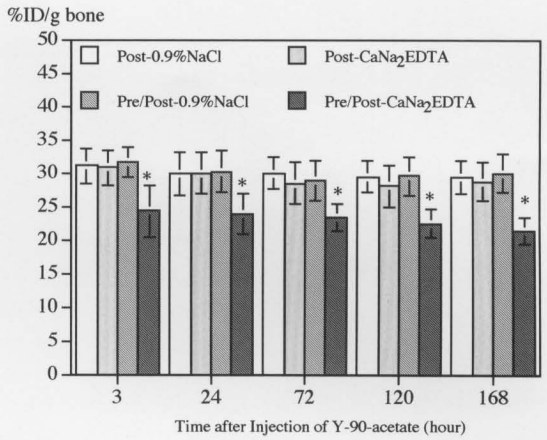


**Fig. 1** Effect of post-administration and pre- and post-administration of $CaNa_2EDTA$ on bony radioactivity in mice with Y-90 acetate. 0.9% NaCl was used as a control. *: $p < 0.05$ compared with the control.

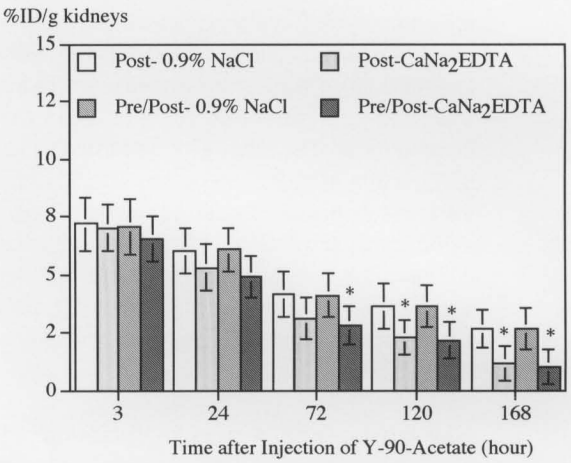


**Fig. 2** Effect of post-administration and pre- and post-administration of $CaNa_2EDTA$ on renal radioactivity in mice with Y-90 acetate. *: $p < 0.05$ compared with the control.

## RESULTS

In the control mice, the radioactivity in the bone had reached a maximum level of approximately 30% ID/g at 3 h post-Y-90 acetate-injection, and the level showed no change for up to 168 h (Fig. 1). The radioactivity in the other normal tissues and blood decreased with time (Figs. 2–4). In the post-$CaNa_2EDTA$-treated mice, no change in the radioactivity of the bone was demonstrated compared to the control mice (Fig. 1). The reduction in Y-90 activity in the kidneys and liver was accelerated, and the radioactivity was significantly lower than the control at 120 h postinjection of Y-90 acetate (Figs. 2 and 3), but not the blood (Fig. 4), spleen, lungs, or muscle (data not shown). In the pre- and post-$CaNa_2EDTA$-treated mice, the radioactivity in the bone at 3 h postinjection was significantly

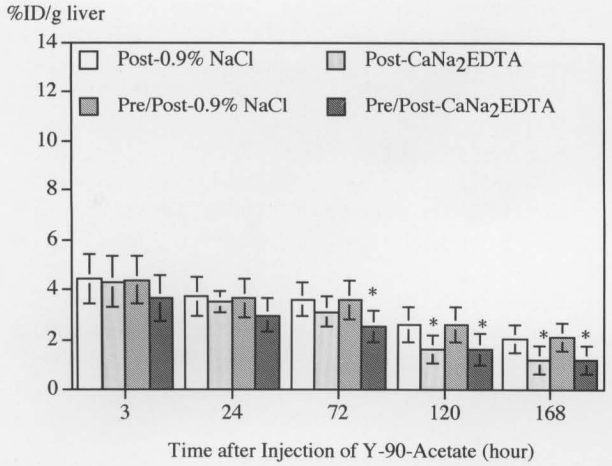


**Fig. 3** Effect of post-administration and pre- and post-administration of $CaNa_2EDTA$ on hepatic radioactivity in mice with Y-90 acetate. *: $p < 0.05$ compared with the control.

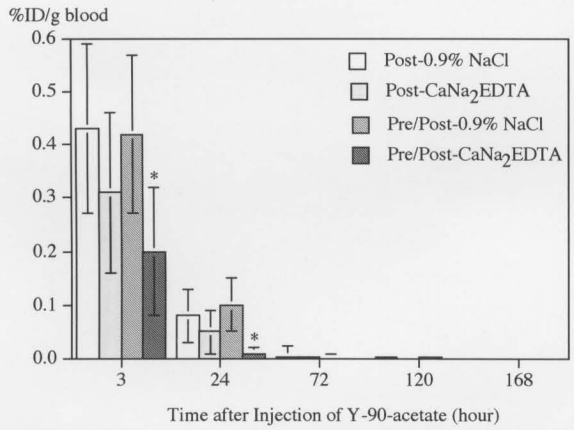


**Fig. 4** Effect of post-administration and pre- and post-administration of $CaNa_2EDTA$ on blood radioactivity in mice with Y-90 acetate. *: $p < 0.05$ compared with the control.

suppressed compared to the control mice (Fig. 1) but the bone radioactivity was not decreased further throughout the study (Fig. 1). The reduction in radioactivity in the blood, kidneys and liver was accelerated, and their radioactivity was lower than in the control at 3 h, 72 h and 72 h postinjection of Y-90 acetate (Figs. 2–4). There was no significantly accelerated reduction in the Y-90-activity in the other tissues (data not shown). No significant difference in the concentration of serum calcium was noted between the $CaNa_2EDTA$-pre- and post-treated mice and the control mice ($4.59 \pm 0.25$ mEq/L vs. $4.60 \pm 0.26$ mEq/L, respectively). There was no inflammation of the abdominal wall in any of the $CaNa_2EDTA$-treated mice. No weight loss or other sign of toxicity was observed. No macroscopic change was observed in the kidneys of the mice.

## DISCUSSION

$CaNa_2EDTA$-treatment has been reported to accelerate the elimination of yttrium-radioactivity in rabbits and to remove yttrium from the skeleton[7] but our study did not demonstrate any chelating effect of $CaNa_2EDTA$ on the radioactivity in the bone of normal mice. There was a significant difference between the doses of $CaNa_2EDTA$ and animal species employed in the two studies; approximately 460 mg/kg of $CaNa_2EDTA$ once or twice a day was given to young rabbits after yttrium (1 h after Y-91 and daily for 9 days) in the former study[7] whereas 37.5 mg/kg of $CaNa_2EDTA$ twice a day was administered to 4-week-old normal mice in the latter (our) study. The dose of 37.5 mg/kg twice a day is the clinically maximum daily dose for children,[11] and the $CaNa_2EDTA$-administration schedule in our study has no influence on serum metallic concentrations such as copper and lead in mice (data not published). Moreover, the introduction of pre-administration of $CaNa_2EDTA$ effectively suppressed the radioactivity in the bone, but even the pre- and post-$CaNa_2EDTA$ treatment could not chelate Y-90 from the bone. Therefore, it is acceptable that the binding of Y-90 to the bone matrix may be too tight to allow chelation by $CaNa_2EDTA$. The pre-administered chelate is speculated to be immediately available to clear free radioactivity. On the other hand, in the post-$CaNa_2EDTA$-treated mice, the radioactivity in the kidneys and liver was reduced. And the pre- and post-administration of $CaNa_2EDTA$ was reduced earlier than with post-administration only. Earlier introduction of $CaNa_2EDTA$-administration was speculated to effectively reduce the radioactivity in the kidneys and liver, however, it is not so well understood how Y-90 distributes at the tissues that the exact mechanism of $CaNa_2EDTA$-mediated reduction in radioactivity is also unclear. In conclusion, the $CaNa_2EDTA$ with the administration schedule employed here cannot chelate the Y-90 from bone but can chelate the Y-90 before deposition into bone. In the pre- and post-$CaNa_2EDTA$-treated nude mice bearing tumors with Y-90 labeled MAb, the radioactivity in the bone may be successfully decreased by removing free Y-90 before its deposition into bone.

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