COMPARTMENTAL REDISTRIBUTION OF ^{99m}Tc-PERTECHNETATE IN THE PRESENCE OF PERCHLORATE ION AND ITS RELATION TO PLASMA PROTEIN BINDING

William H. Oldendorf, Warden B. Sisson and Youichi Iisaka

Wadsworth Hospital, Veterans Administration Center, Los Angeles, California and UCLA School of Medicine, Los Angeles, California

This report is concerned with studies of the effects of prior administration of perchlorate (ClO_4^-) ion upon the distribution of pertechnetate ($^{99m}TcO_4^-$) ion in certain tissues of the rabbit. These studies document the well-recognized perchlorate suppression of choroid plexus concentration of pertechnetate noted earlier during clinical brain scanning (*1,2*). When perchlorate was added, an unexpected increase in tissue content of TcO_4^- relative to plasma was observed in all other tissues studied here. This observation led to a series of experiments intended to define various parameters of this redistribution. These changes in distribution appear to be unreported previously.

METHODS AND MATERIALS

White New Zealand rabbits, weighing 3–4 kg, were given intravenously 100–200 μCi of ^{99m}Tc-pertechnetate obtained from a commercial ^{99}Mo cow. Thirty minutes later the animals were decapitated after receiving 80–100 mg sodium pentobarbital intravenously. Specimens of scalp, lumbar skin, cranial skull, the entire brain, lumbar paraspinous muscle and heparinized cardiac blood were obtained. The choroid plexus of both lateral ventricles was also dissected out. The specimens were placed in pre-weighed test tubes, capped to prevent evaporation and post-weighed. The cutaneous specimens were taken from areas clipped free of hair. The ^{99m}Tc content of each tissue specimen was measured in a gamma well counter. All cardiac blood specimens were centrifuged immediately and plasma separated.

In addition to these nine control animals, nine experimental animals received 3 mg/kg of potassium perchlorate ($KClO_4$) intravenously, immediately prior to injection of ^{99m}Tc-pertechnetate. Tissue specimens were taken as in the control group.

During preliminary studies it was noted that ClO_4^- caused an apparent TcO_4^- shift intracellular in blood with a marked change in plasma-to-red cell ratio. In four animals the perchlorate dose dependence of this shift into red cells was studied. Approximately 50 μCi of $^{99m}TcO_4^-$ was injected intravenously with 8 μCi ^{125}I-human serum albumin (IHSA). Although the IHSA used here contained less than 0.5% dialyzable iodide ion, it was felt necessary to reduce this substantially. It was maintained essentially iodine-free by storage of the solution with sterile anion exchange beads (AG^R 1–X8, 50–100, chloride form, Bio-Rad Laboratory, Richmond, Calif.). After 10 min post-injection, complete equilibration of plasma and of extracellular fluid was assumed. A cardiac blood sample was obtained, and the lowest dose (0.5 mg) of $KClO_4$ was injected intravenously. After 5 min another cardiopuncture specimen was obtained and an additional dose of $KClO_4$ was injected i.v. This was repeated until the perchlorate dose range was from 0 to 52.6 μM/kg of rabbit. The rapidity of the intracellular shift of the TcO_4^- was determined in one animal by drawing serial blood specimens after intravenous injection of perchlorate.

To find the plasma-to-red cell ratio of ^{99m}Tc, the heparinized cardiopuncture specimens were immediately centrifuged and most of the plasma removed to another pre-weighed test tube. Both the remaining packed red cells and the plasma were counted for ^{99m}Tc and ^{125}I. After correction for plasma content of the incompletely packed red cells, the plasma-to-red cell ^{99m}Tc concentration ratio was calculated. The ^{125}I was assumed to be restricted to plasma and to be uniformly distributed therein.

To determine whether or not there was some effect of general extracellular fluid TcO_4^- redistribution causing the shift into red cells, the ClO_4^-

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For reprints contact: William H. Oldendorf, Veterans Administration Center, Wilshire and Sawtelle Blvds., Los Angeles, Calif. 90073.

TABLE 1. ^{99m}Tc-PERTECHNETATE TISSUE SPACES 30 MIN AFTER INJECTION*

Tissues	Plasma	Scalp	Lumbar skin	Muscle	Skull	Brain	Choroid plexus†
Without $KClO_4$	100.0	36.53	32.87	4.52	12.58	0.85	126.95
S.D.		(4.66)	(4.35)	(0.45)	(2.93)	(0.19)	(15.99)
With $KClO_4$	100.0	50.53	47.23	5.99	18.50	1.11	25.86
S.D.		(9.34)	(3.03)	(1.14)	(3.14)	(0.25)	(7.24)
p factor‡		<0.001	<0.001	>0.001 <0.01	<0.001	>0.02 <0.05	<0.001

* Nine animals were injected with 3 mgm/kg of $KClO_4$, 5 min before injection of technetium and decapitated at 30 min; the above six terminal tissue specimens were obtained. Nine animals not receiving $KClO_4$ served as controls. Three minutes before decapitation all animals received 8 μCi of intravenous ^{125}I-iodinated human serum albumin (IHSA). All tissue samples were corrected for plasma content through the use of this IHSA.

† Four animals were used from each group of nine for the choroid plexus determination.

‡ p factor expresses the probability that the treated and untreated tissue differences are random.

TABLE 2. CHLORIDE DISTRIBUTION IN WHOLE BLOOD AND PLASMA (Blood Cl)/(Plasma Cl) AFTER I.V. INJECTION OF PERCHLORATE

Experiment*	0 min	5 min	1 hr	2 hr	4 hr	24 hr
With $KClO_4$†						
1	0.76	0.76	0.75	0.72	0.75	0.77
2	0.75	0.75	0.76	0.77	0.74	0.80
Without $KClO_4$						
3	0.76	0.78	0.74	0.72	0.74	0.77
4	0.78	0.78	0.76	0.77	0.75	0.77

* Each experiment represents one rabbit, and all specimens were cardiac blood samples.

† ClO_4^- 21.6 μM/kg rabbit were given intravenously immediately after 0 min specimen.

effect was tested *in vitro*. Blood from the heart of an animal in which ^{99m}Tc-pertechnetate and ^{125}I-IHSA had been injected intravenously 20 min earlier, was first drawn into a syringe containing heparin, and a second 5-ml cardiac specimen was drawn into another syringe containing 0.5 mg $KClO_4$. Heparin was added to the second specimen after blood and $KClO_4$ had been mixed. The plasma and red cell concentrations were calculated from ^{99m}Tc and ^{125}I counts as described earlier.

The chloride content of blood and plasma of two perchlorate-treated and two untreated control animals was determined by the electrodeposition method of Cotlove (*3*) to determine whether the intracellular shift of TcO_4^- was accompanied by a chloride shift.

To estimate the ability of ClO_4^-, in the dose range studied here to interfere with protein binding of TcO_4^-, a dialysis experiment was performed. Blood specimens were obtained from four rabbits, eight rats and three humans. The blood was drawn directly into syringes containing heparin and about 50 μCi of $^{99m}TcO_4^-$. Plasma was separated by centrifugation and 1 ml was placed in dialysis tubing [regenerated cellulose dialyzer tubing, 0.25 in. (6.3 mm)] tightly ligated at both ends. This was placed in a sealed bottle containing 10 ml of Ringer's solution and gently shaken for 2 hr at 37°C. Equilibration of the inside unbound TcO_4^- and the outside TcO_4^- was essentially complete since continuation to 3 hr did not substantially alter the relative concentration. By comparing the inside and outside ^{99m}Tc concentrations, the excess concentration inside was calculated. This inside excess constituted the nondialyzable fraction. The nondialyzable percentage is

$$\frac{\text{Concentration inside} - \text{concentration outside}}{\text{Concentration inside}} \times 100.$$

This experiment was repeated with progressively greater amounts of $KClO_4$ added to the outside compartment.

RESULTS

The effect of perchlorate ion on $^{99m}TcO_4^-$ distribution is to increase significantly the ^{99m}Tc content relative to blood plasma of scalp, lumbar skin, skull, muscle and brain 30 min after injection. Choroid plexus isotope content is greatly diminished. Tissue isotope concentration with and without ClO_4^- is shown relative to that of blood plasma in Table 1 and Fig. 1.

The dose dependence of the effect of perchlorate on the plasma-to-red cell TcO_4^- ratio is shown in

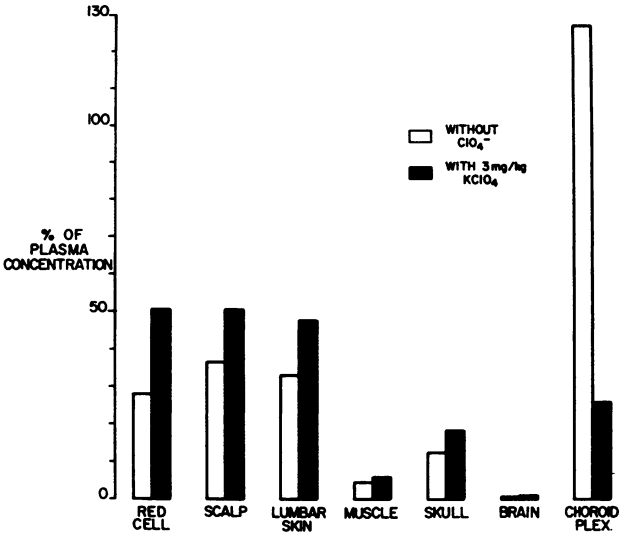

FIG. 1. When perchlorate is present, tissue pertechnetate space is larger for all tissues studied other than choroid. Numerical data and their statistical analyses are in Table I.

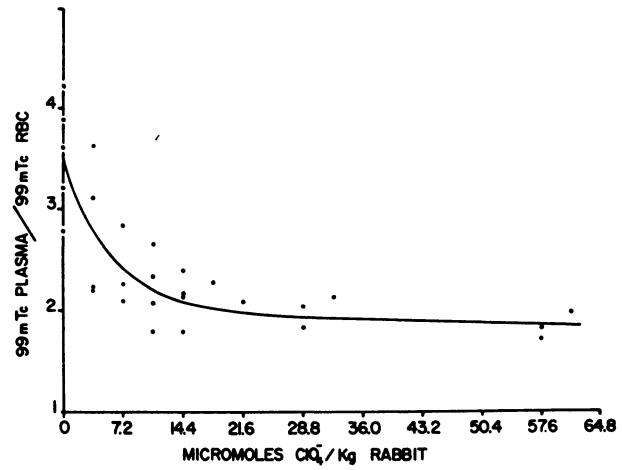

FIG. 2. Shows dose dependence of shift in plasma-to-red cell concentrations with intravenous administration of increasing amounts of $KClO_4$. In text dose refers to mg/kg animal weight. One milligram of $KClO_4$ is equivalent to 7.2 μM perchlorate ion.

Fig. 2. Beyond about 2 mg/kg $KClO_4$ (14.4 μM/kg ClO_4^-) only a minimal additional effect is seen. Figure 3 shows the rapidity of the intracellular shift of $^{99m}TcO_4^-$ in the presence of perchlorate.

The *in vitro* specimens showed a plasma-to-red cell ratio in the absence of perchlorate of 3.51 and 1.25 with perchlorate.

There was no measurable difference between the red cell and plasma distribution of chloride in the treated and untreated animals (Table 2).

The results of the dialysis experiment are shown in Fig. 4. The difference between rat and rabbit is of questionable significance. The difference in release of TcO_4^- from human plasma protein relative to rat and rabbit is highly significant.

DISCUSSION

A part of the tissue increase relative to plasma must be due to release of TcO_4^- from plasma protein binding sites into free solution and subsequent redistribution of this unbound TcO_4^-. These data indicate a competition of ClO_4^- for TcO_4^- protein binding sites with release of bound TcO_4^-. This release occurs within the plasma concentration range studied here *in vivo*. This unbound TcO_4^- will, after release from protein, equilibrate with the extracellular fluid of the various tissues.

Another factor which may partially explain the observed general tissue increase is a greater access of TcO_4^- to the intracellular compartment in the

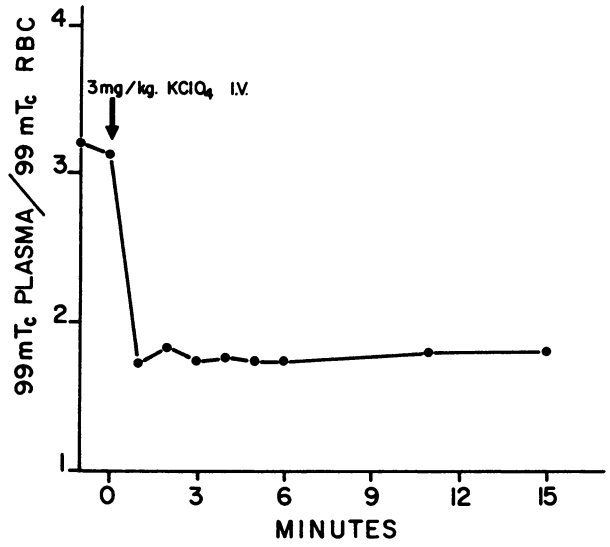

FIG. 3. Indicates rapidity of redistribution of red cell vs. plasma TcO_4^- after intravenous injection of perchlorate.

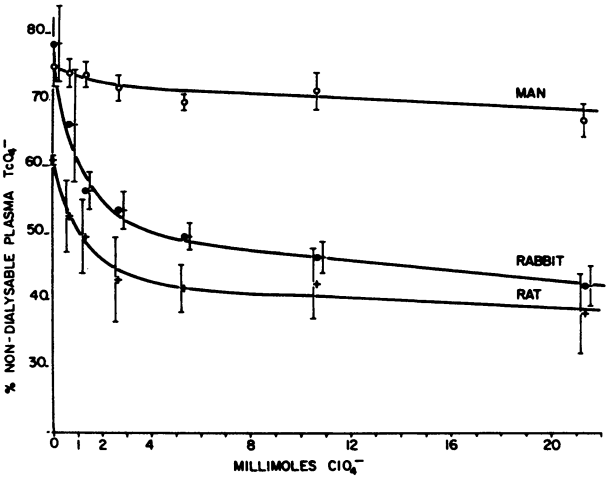

FIG. 4. Results of dialyzing 1 ml of plasma containing TcO_4^- against 10 ml of Ringer's solution for 2 hr at 37°C. Variable amounts of ClO_4^- were added to Ringer's solution to study dose dependence of release from binding. Three curves represent 77 dialyses performed on 11 plasma specimens.

presence of ClO_4^-. The intracellular shift demonstrated here in blood may also occur in other tissues as well. This intracellular shift may also be due in large part to release of TcO_4^- from plasma protein binding sites into free solution in plasma from which it can more readily diffuse through plasma membranes.

It is also possible that TcO_4^- is continually extruded from cell surfaces by some pump mechanism maintaining the concentration gradient between inside and out. The effectiveness of this pump mechanism for TcO_4^- may be suppressed by ClO_4^- competition.

Although there is considerable relative redistribution of TcO_4^- between tissue compartments in the presence of ClO_4^-, the absolute amount of isotope in any anatomical region will not change greatly. Thus no visible change in clinical scanning appearance is anticipated in the presence of ClO_4^- except suppression of active uptake by such organs as thyroid, parotid and choroid plexus. The slight difference in dialyzability of TcO_4^- in man with ClO_4^- would further suggest a minimal clinical effect. It could be anticipated that those organs actively concentrating TcO_4^- will show a visible suppression of uptake in the presence of ClO_4^-.

Additional studies will be required to further clarify the mechanisms involved in the findings reported here. It would be of interest to compare the dose dependence of the intracellular shift into red cells and the release of TcO_4^- from plasma protein binding. If both phenomena showed saturation at the same concentration, it would suggest that all of the general tissue compartmental shifts noted in this study are secondary to TcO_4^- release from plasma protein binding sites by ClO_4^-.

The values for the TcO_4^- space in muscle shown in Table 1 are considerably below the inulin, sucrose and raffinose space (about 10%) described by others (*4,5*). The reasons for this remain unclear, but this too may represent a considerable residual plasma protein binding of TcO_4 even in the presence of ClO_4^-. These studies indicate that TcO_4^- is a poor tracer for quantitative extracellular space studies since it is largely protein bound in plasma, even in the presence of ClO_4^-.

SUMMARY

It was shown in rabbits that the addition of 3 mg/kg of perchlorate (ClO_4^-) ion results in a major tissue redistribution of pertechnetate (TcO_4^-). This probably results from (1) release of TcO_4^- from plasma protein binding sites with resulting redistribution to tissue extracellular spaces and (2) a shift of a portion of the TcO_4^- intracellularly. The injection of this dose of ClO_4^- results in tissue levels relative to blood plasma about 1.38 times greater than without ClO_4^-.

In the presence of ClO_4^-, TcO_4^- moves from plasma into red cells. The ClO_4^- dose dependence of this plasma-to-red cell shift was studied *in vivo*. The ratio of plasma-to-red cell concentration of TcO_4^- shifts from 3.98 ± 0.31 without ClO_4^- to 1.98 ± 0.32 with 3 mg/kg of ClO_4^-. This intracellular shift was demonstrated *in vitro* at a single high concentration of ClO_4^- producing a ratio of 1.25.

The effect of ClO_4^- on plasma-to-red cell distribution is complete within 1 min of i.v. injection in the rabbit.

Chloride does not redistribute between red cells and plasma as does TcO_4^- in response to ClO_4^-.

Preliminary dialysis studies indicate about 80% of plasma TcO_4^- is protein bound in rabbit and man. This drops to about 40% in the presence of ClO_4^- concentrations used in this study in the rabbit but changes very little in man.

The tissue compartmental redistribution in the presence of ClO_4^- described here is not expected to produce visible changes in clinical scan appearance except in those organs where active uptake is present.

Possible mechanisms responsible for this redistribution are discussed.

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