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Establishment of Tissue Biodistribution and Blood Clearance Rates of Intravenously Administered Radioactive $^{51}Cr^{3+}$ in New Zealand White Rabbits

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HIGHLIGHTS

- Tissue biodistribution of intravenously administered trivalent chromium.
- Blood clearance rates of intravenously administered trivalent chromium.
- Faster systemic blood clearance is through renal excretion.
- Understanding of the above parameters is relevant for its diagnostic applications.

Abstract: Radioactive trivalent chromium ($^{51}Cr^{3+}$) is a known radiopharmaceutical used to tag plasma proteins, platelets and also for estimation of blood volume. Nevertheless, there exist insufficient reports with limited sample sizes concerning its clearance from blood and its biodistribution after intravenous administration. This study focused to understand clearance rate of $^{51}Cr^{3+}$ from blood and analyze its biodistribution. For biodistribution, six adult New Zealand white albino rabbits were injected with $^{51}Cr^{3+}$ through their marginal vein. Percentage clearance of $^{51}Cr^{3+}$ from blood was calculated by recording

radioactive counts obtained at 1, 58, 61, 120, 180 and 240 minutes post-administration in thirty-three adult New Zealand white albino rabbits. For evaluating $^{51}Cr^{3+}$ biodistribution, organs were surgically removed from the rabbits and weighed. Radioactivity of the organs and urine were counted in a nucleonix gamma-ray spectrometer with a NaI scintillation detector. Data were expressed as cps/g. Average clearance of $^{51}Cr^{3+}$ was 34% from the first to the 58 minute. Subsequent measurements for hourly clearance at 120, 180 and 240 minutes showed percentage reduction of radioactivity of 33, 14 and 8, respectively. Minimal specific activities were found in the muscle and brain. Spleen, lungs, liver and kidneys exhibited moderate radioactivity. Urine tracer-concentrations were found to be ten times more than that of plasma. From this study, it has been observed that clearance of $^{51}Cr^{3+}$ from blood was faster initially which slowed down progressively and there displayed moderate uptake of $^{51}Cr^{3+}$ by certain organs. Understanding pharmacokinetics of $^{51}Cr^{3+}$ is relevant for its potential use as a diagnostic tool.

Keywords: Trivalent chromium; Biodistribution; blood volume; New Zealand white rabbits; blood clearance.

INTRODUCTION

The utility of radioactive chromium for plasma volume measurements was recognized as early as 1950 [1]. Both, hexavalent and trivalent forms of chromium ($^{51}Cr^{3+}$) were employed as radiotracers for simultaneous clinical measurements of total red blood cell mass and plasma volume, in man [2]. It was observed that red cells did not imbibe $^{51}Cr^{3+}$ following its intravenous (IV) injection; instead, $^{51}Cr^{3+}$ had entirely become bound to the plasma proteins [1, 2]. This property of $^{51}Cr^{3+}$ permitted its utility for the measurement of plasma volume [3]. These observations eventually paved the way to determine blood volume.

There exist reports of $^{51}Cr^{3+}$ being used for direct blood volume measurement, check the intactness of the blood-brain barrier, brain tumor localization and quantifying gastrointestinal protein loss [4-7]. However, a detailed evaluation of tissue biodistribution and tissue-toxicity-profiling of $^{51}Cr^{3+}$ is obligatory before ascertaining its utility and safety in clinical practice. There are only a few articles that confer information for the same which are based on experiments in rats, dogs, sheep and rabbits without appropriate mentioning of the species of the animals. Another drawback observed was that all these studies were of smaller sample sizes that involved multiple subgroups, providing less reliability to the results [1, 8-10].

Earlier, we had reported a novel method for the measurement of blood volume in rabbits which required the aid of a correction factor. To estimate the correction factor critical parameters like blood clearance, organ biodistribution and renal excretion rate were determined [4, 11].

In this article, we report data on the tissue biodistribution and blood clearance rates of intravenously administered radioactive $^{51}Cr^{3+}$ in New Zealand white rabbits which was previously unreported during the studies on blood volume determination using $^{51}Cr^{3+}$.

MATERIAL AND METHODS

The institutional animal ethics committee approved the study. A total of thirty-three New Zealand white rabbits (*Oryctolagus cuniculus*) which include twenty-two females and eleven males weighing approximately 1.5 – 3.1 kg were used for the understanding of clearance of $^{51}Cr^{3+}$ from the blood. Out of the thirty-three, six New Zealand white rabbits, three males and three females, were used for the biodistribution part of the study. All rabbits were fed with food and water ad libitum. During the experimental procedure, rabbits were kept on dietary restriction to prevent any fluctuations in blood volume. Rabbits were placed in a rabbit restrainer and the sites for injection and blood collection on both ears were prepared by shaving and wiping the spots with 70% alcohol. Ten minutes prior to the experiment, rabbits were anesthetized with ketamine hydrochloride injection (25 mg/kg body weight) through the marginal ear vein.

A 26G intravenous cannula (BD Neoflon^{TM}) was inserted into the marginal ear vein of the anesthetized rabbits and secured with adhesive plaster. Drugs and the radiopharmaceutical were injected through this route. Into the auricular artery of the other ear of the rabbits, a 24G intravenous cannula (BD Neoflon^{TM}) was inserted which was then secured with adhesive plaster. Blood was drawn out from this cannula [12].

Two mCi of ^{51}Cr in aqueous solution was obtained from BRIT, DAE and GOI. Two syringes were loaded, both with one mL of solution that contained ^{51}Cr and freshly prepared ascorbic acid (Sisco) (mass concentration of ascorbic acid = 2mg/mL). A two-hour holding time was given to ensure the complete reduction of chromium to $^{51}Cr^{3+}$ [13]. Radioactivity in the loaded syringes was measured in a calibrated gamma-ray spectrometer with thallium-doped sodium-iodide (NaI (TI)) well-type scintillation detector coupled to a single-channel analyzer. All records of radioactivity were counted for thirty seconds and further converted to counts per second (cps) for calculations.

The clearance rate of $^{51}Cr^{3+}$ from blood at the first minute to 58th minute following initial dose

Approximately 3000 cps of $^{51}Cr^{3+}$ in one mL was injected through the IV cannula in the marginal vein which was followed by a 0.5 mL normal saline. Thereafter, residual activity was measured from this syringe. The injected $^{51}Cr^{3+}$ was calculated after deducing the left-over activity in the loaded syringe. One minute subsequent to injection, one mL blood sample was collected from the IV cannula in the auricular artery. This sample was obtained for blood volume calculation. At the 58th minute following $^{51}Cr^{3+}$ injection, another 1 mL blood sample was drawn and its radioactivity measured. The percentage clearance of $^{51}Cr^{3+}$ was calculated using the following formula:

$$\frac{cps1 - cps58}{cps1} \; x \; 100$$

cps1 = counts obtained from one mL blood sample drawn at one minute post $^{51}Cr^{3+}$ injection.
cps58 = counts obtained from one mL blood sample drawn at 58th minute post $^{51}Cr^{3+}$ injection.

The clearance rate of $^{51}Cr^{3+}$ from blood following a spike dose

Ensuing the above procedure, a repeat/second dose of approximately 3150 cps of $^{51}Cr^{3+}$ was injected through the marginal vein followed by a 0.5 mL saline wash at the 60th minute. After this spiking dose, one mL blood sample was obtained at the 61st minute to measure the radioactivity. This was followed by collection of one mL blood sample each at 120, 180 and 240 minutes post first dose of $^{51}Cr^{3+}$ injection for radioactivity measurement, in cps/mL, for calculation of clearance kinetics. Percentage clearance was obtained using the same formula mentioned above.

Biodistribution study of $^{51}Cr^{3+}$ in organs of sacrificed rabbits

At the end of four hours post $^{51}Cr^{3+}$ injection, six rabbits (three males and three females) were euthanized by an overdose administration of ketamine hydrochloride intravenously. The organs were removed surgically and weighed in a pre-weighed container. A small section was cut from each of the organs and was placed in a pre-weighed 1.5 mL microcentrifuge tube. The organ-section was then weighed and its radioactivity measured.

The bladder was completely drained and the urine weighed. One mL urine sample, measured by weight, was obtained from the total volume of urine.

Radioactivity of different organs as well as urine was counted and the data was expressed as cps/g of tissue using the following formula:

$$cps\ per\ g\ of\ tissue = \frac{counts\ per\ g}{360}$$

RESULTS

The amount of $^{51}Cr^{3+}$ in blood decreased over time post its intravenous administration. The average clearance of $^{51}Cr^{3+}$ was 34% from the first to the 58th minute. Clearance of $^{51}Cr^{3+}$ from blood after the first dose is displayed in Figure 1.

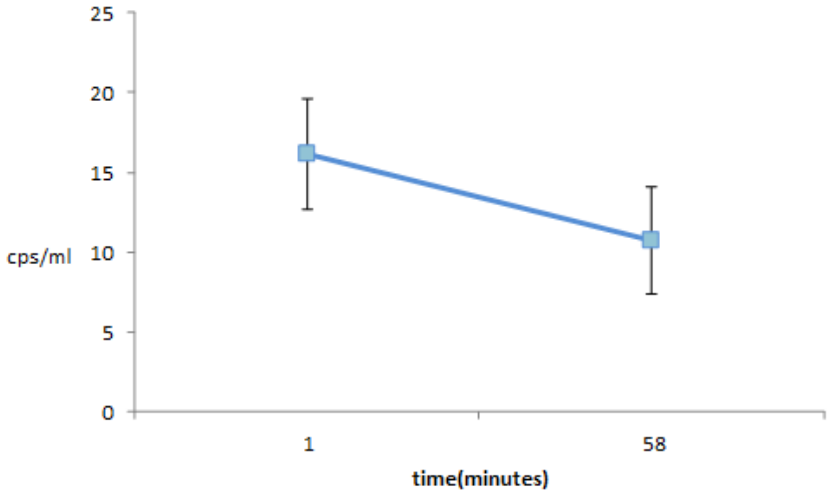

Figure 1. Clearance rate of $^{51}Cr^{3+}$ from blood after intravenous administration of initial dose.

Figure 2 exhibits increase in radioactivity-counts by 1.72 times in blood owing to the spike dose of $^{51}Cr^{3+}$ at the 60th minute post first dose. Subsequent measurements of counts for hourly clearance from blood at 120, 180 and 240 minutes indicated a percentage reduction of radioactive-counts of 33, 14 and 8, respectively. Counts are expressed in cps/mL. With time there appeared a reduction in rate of clearance of $^{51}Cr^{3+}$.

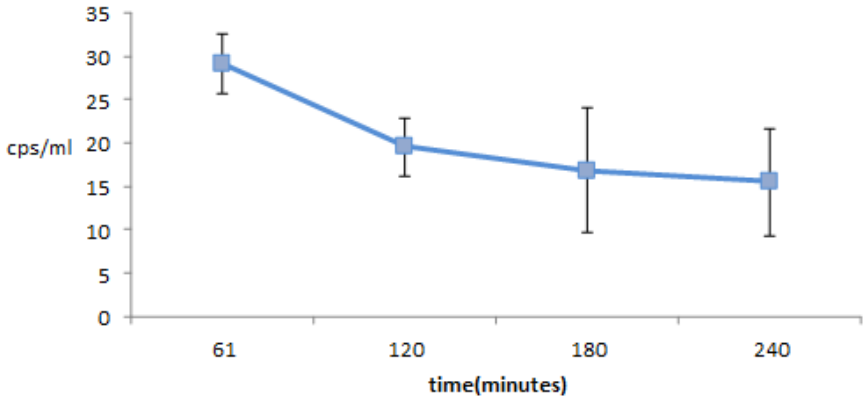

Figure 2. Clearance rate of $^{51}Cr^{3+}$ from blood following intravenous administration of spike dose.

Radioactivity in the different organs was measured at the 240th minute (four hours) following the first dose of $^{51}Cr^{3+}$injection. This measurement reflects the cumulative effects of both the first as well as the spike doses, amounting to approximately 6150 cps. The data belonging to the parametric type was expressed in mean ± SD and in median and inter quartile range (IQR) for those measurements that obeyed the non-parametric pattern. The radioactivity counts of all organs were observed to be lesser than that of blood. Minimal specific activities were exhibited by the muscle and brain tissues. Tissues of the heart, bone, lymph node, diaphragm, stomach, ovaries, pancreas, epididymis, submandibular gland and testis retained diminutive amounts whereas the spleen, lungs, liver and kidneys presented moderate radioactivity. Maximum radioactivity was obtained in the urine samples. Urinary tracer concentrations were found to be exceeding ten times that seen in plasma. Table 1 shows the estimated counts in the various tissues.

Table 1. Distribution of ^{51}Cr (III) after intravenous injection.

Organs	No. of rabbits	cps/g of tissue (Mean ± SD)
Blood	5	11.84±3.69
Heart #	6	3.07(2.40)
Spleen #	6	5.94(14.49)
Lymph node #	5	3.72(1.99)
Lungs #	6	7.84(7.76)
Diaphragm #	5	1.11(1.52)
Muscle	5	0.92±0.34
Bones	6	3.23±1.13
Brain #	5	0.27(0.17)
Liver #	6	6.15(1.61)
Pancreas #	3	1.95(1.26)
Stomach #	5	0.75(4.72)
Submandibular gland	3	1.69±0.82
Kidneys	6	8.71±4.17
Urine	5	139.30±23.40
Testis	2	1.8(0.87)
Epididymis	3	2.79±0.95
Ovaries #	3	0.47(4.50)

# indicates Median & IQR

DISCUSSION

Clearance of $^{51}Cr^{3+}$ from circulation was observed at multiple time-points following the administration of two doses of $^{51}Cr^{3+}$. The percentage clearance of $^{51}Cr^{3+}$ that was observed at the 58th minute, subsequent to the first dose of approximately 3000cps of $^{51}Cr^{3+}$, was 34%. Owing to its high affinity to form colloid complexes in plasma, levels of $^{51}Cr^{3+}$ were found to be high in blood [3]. Following the spike dose administration of $^{51}Cr^{3+}$ at the 60th minute following the first dose, percentage clearance of $^{51}Cr^{3+}$ observed at 61, 120, 180 and 240 minute appeared to be diminishing with values 33,14 and 8, respectively. This tapering clearance of $^{51}Cr^{3+}$ attributes to the flux of the tracer across capillaries, its loss through urine, its excretion through the gut and also its flux out of the tissues. Kraintz and Talmage described in their study the determination of radioactivity distribution in rabbits following intravenous administration, wherein it was suggested that the gradual and decreased clearance of $^{51}Cr^{3+}$ occurred due to its deposition in the reticuloendothelial system [9]. In our study, it is clear that the clearance of dual-dosed $^{51}Cr^{3+}$ from blood is independent of the number of dose administered. Similar studies on rats showed that the faster initial clearance is attributed to the loss of tracer through urine whereas the slow clearance observed in the later hours is due to the reduced release of chromium from the reticuloendothelial system [8]. This study correspondingly reports a rapid clearance rate of chromium in the initial hours that diminishes in the later hours.

In addition to determining the circulatory $^{51}Cr^{3+}$ clearance rate, we also attempted to investigate the biodistribution of $^{51}Cr^{3+}$ in various organs, four hours post $^{51}Cr^{3+}$ intravenous administration of two doses, which totally amounted to about 6150 cps. It appeared that different organs exhibited varying amounts of $^{51}Cr^{3+}$ retention. However, radioactivity measured in each tissue was lesser when compared to that obtained from blood [5]. Moderate $^{51}Cr^{3+}$ uptake was exhibited by the spleen, lungs, liver and kidneys. Minimal activity was presented by the muscle and brain attributable to the inability of $^{51}Cr^{3+}$ to penetrate the muscle membrane and the blood-brain barrier respectively [5]. Tissues of the heart, bone, lymph nodes, diaphragm, stomach, ovaries, pancreas, epididymis, submandibular gland and testis retained a minute amount of radioactivity. The study conducted by Hopkins indicated a higher $^{51}Cr^{3+}$ uptake by mature testis of rats. On the contrary, our results in rabbits did not show significant $^{51}Cr^{3+}$ uptake [8]. Urinary tracer concentrations were found to be ten times more than that recorded from the plasma. In a similar study, Edstrom observed that one-third of the dose given intravenously to rabbits was excreted in three days [5]. There are limited studies that describe biodistribution using $^{51}Cr^{3+}$ in rabbits [9,10]. Also, existing studies fail to mention the type of rabbit species involved in the experiments. This makes it challenging to rely on available data for initiating newer studies. Moreover, a proper comparison of data is difficult to achieve.

An unexpected downfall we encountered during the procedure was that there occurred radioactive contamination among few organs during the dissection process. Such organs were discarded and not included in the study and hence a disparity in the number of organs harvested. Repeat experimentation on fresh rabbits to equalize the sample size was not possible due to ethical constraints. Therefore, in future studies, we advise that such issues like contamination of organs during dissection along with animal attrition have to be looked into while planning experiments on biodistribution priorly.

CONCLUSION

Understanding the pharmacokinetics of $^{51}Cr^{3+}$ is relevant for its potential use as a diagnostic tool. $^{51}Cr^{3+}$ has gained popularity in experimental research in fields of blood volume measurements and in experiments that required to verify the intactness of membrane barriers. However, a proper comprehension of its biodistribution is necessary for its utility in the medical domain. This study establishes specific details regarding it's clearance that was deciphered in real-time and also displays its biodistribution.

$^{51}Cr^{3+}$ clearance from blood occurred at a biphasic manner, exhibiting rapid clearance initially and that slowed down progressively. Moderate uptake of $^{51}Cr^{3+}$ was presented by certain organs, especially by those that housed the reticuloendothelial system. Radioisotopes hold great promise in therapeutics and diagnostics provided its clearance and biodistribution are understood.

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Conflicts of Interest: "The authors declare no conflict of interest."

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