Preparation of ^{99m}Tc Labeled Vitamin C (Ascorbic Acid) and Biodistribution in Rats

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The aim of this study was to label ascorbic acid with ^{99m}Tc and to investigate its radiopharmaceutical potential in rats. Ascorbic acid was labeled with ^{99m}Tc using the stannous chloride method. The radiochemical purity of [^{99m}Tc]ascorbic acid (^{99m}Tc-AA) was determined by RTLC, paper electrophoresis, and RHPLC methods. The labeling yield was found to be 93±5.0%. The maximum labeling yield of ^{99m}Tc-AA was determined at pH 5 and 25ºC. The biodistribution studies related to ^{99m}Tc-AA were done in male albino Wistar rats. ^{99m}Tc-AA, which has a specific activity of 13.02 GBq/mmol, was administered into the tail vein of the rats. The rats were sacrificed at 15, 30, 60, and 120 min after the injection by heart puncture under ether anaesthesia. The organs were weighed after removal. Their activities were counted using a Cd(Te) detector equipped with a RAD 501 count system. The %ID/g (% of injected dose per gram of tissue weight) in each organ and in blood was calculated. Maximum uptake of ^{99m}Tc-AA was observed in prostate and kidneys at the 60th min. ^{99m}Tc-AA may be a promising radiopharmaceutical for the imaging of prostate and kidneys.

Key words ascorbic acid; ^{99m}Tc-ascorbic acid (AA); vitamin C

Vitamin C (ascorbic acid) (Fig. 1) is a water soluble vitamin that possesses a variety of physiological properties. Ascorbic acid (AA) is an essential micronutrient involved in many biologic and biochemical functions.[1—5] It is an excellent antioxidant in biological systems such as α-tocopherol, and β-carotene.[4] The known functions of AA are accounted for by its action as an electron donor, or reducing agent.[6—8] Its most significant role is seen to be as a reductant that minimizes damage by oxidative processes along with other natural antioxidants.[9] AA seems to affect many enzyme activities and physiologic processes.[1,2,6—8,10] AA efficiently neutralizes reactive oxygen and nitrogen species such as hydroxyl, peroxyl, and super oxide radicals, as well as peroxynitrite, nitroxide radicals, single oxygen, and hypochlorite. This action provides direct antioxidant protection to tissues subjected to high free-radical stress such as phagocytes, eye, brain, stomach, and sperm.[11,12]

The biological basis of recommendation for humans should account for absorption and disposition of ascorbic acid which is governed by the following factors: bioavailability and absorption in the gastrointestinal tract, concentration in the circulation, tissue distribution, excretion, and metabolism. Fundamental to all of the issues are the principles of ascorbate chemistry and mechanisms of membrane transport and cellular accumulation.[4] The ascorbate oxidation product; dehydroascorbic acid, is reduced by cells to ascorbate. AA exists in the body in its reduced form. Humans cannot synthesize AA, it must be provided exogenously in the diet and transported intracellularly.[1—3,5,13] There are potential pharmacological uses of AA, such as for cataracts, coronary heart disease, cancer, Parkinson's disease, Alzheimer disease, and the common cold.[14,15]

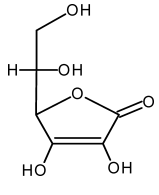

Fig. 1. Structure of Ascorbic Acid

Many studies were carried out on the metabolism of AA by using AA or labeled AA with different radionuclides (^{3}H, ^{14}C, ^{18}F, *etc.*).[16—23]

The metabolism of ascorbic-1-[^{14}C] acid in experimental human scurvy was investigated by Baker *et al.*[17] Tolbert *et al.* studied the metabolic fate of AA in man using L-ascorbic 4-[^{3}H] acid.[18] Wright *et al.* measured the intercellular level of ascorbate in isolated rat lung cells and the concentration in rat plasma and the membrane transport properties for ascorbate influx in these cells.[20] Vera *et al.* analyzed the kinetics of the uptake of dehydroascorbic acid by HL-60 cells under experimental conditions that enabled the differentiation of dehydroascorbic acid transport from the intracellular reduction/accumulation of ascorbic acid by using L-[^{14}C]ascorbic acid.[13] Agus *et al.* identified the chemical form of AA that readily crosses the blood-brain barrier, and the mechanism of this process using [^{14}C]ascorbic acid.[16] 6-Deoxy-6-[^{18}F]fluoro-L-ascorbic acid was synthesized and in rats biodistribution was investigated by Yamamoto *et al.*[9] However, there have been no studies, regarding the labeling of AA with ^{99m}Tc.

The aim of the present study was to investigate the radiopharmaceutical potential of ^{99m}Tc-AA in rats.

Experimental

All chemicals were obtained from Merck. Na^{99m}TcO$_4$ was supplied by the Department of Nuclear Medicine of Ege University, as ^{99}Mo/^{99m}Tc generator eluent (Monrol, Turkey). Cellulose acetate plates were purchased from Merck and used for radio thin layer chromatography (RTLC). RTLC was carried out in a Sigma ITLC tank. Plastic plates (20×20 cm, thickness, 0.1 mm) were cut into 10×1.5 cm sheets. ACD, [0.068 M citric acid, 0.074 M dextrose 1/1 (v/v) (pH 5) was adjusted with 0.1 M NaOH)] and serum physiologic as eluent were used for developing. After developing, the sheets were dried and covered by cello-band. They were cut into 0.5 cm strips and the activities were counted with a Cd(Te) detector equipped with a RAD 501 sin-

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gle channel analyzer.

Paper electrophoresis was performed using a Gelman electrophoresis chamber. Cathode and anode poles and application points were indicated on Whatman papers and the papers were moistened by ACD buffer solution. They were put into the electrophoresis chamber after the samples were applied to strips. The standing time and voltage were 55 min and 250 V, respectively. They were dried and cut into 1 cm strips and counted using a Cd(Te) detector equipped with a RAD 501 single channel analyzer.

HPLC analysis was performed on a Shimadzu HPLC (LC-10 ATvp) equipped with an SPD 10Avp UV detector, quaternary pump, a 1 ml loop, and Cd(Te) detector equipped with a RAD 501 single channel analyzer. An analytical RP-C18 column (Macherey-Nagel, EC150/4.6 Nucleosil 100-3C-18) was used, the flow rate was adjusted to 1 ml/min of mobile phase; [25 mM KH_2PO_4 (pH 3) and methanol (96/4 : v/v)].[6,21] UV detection was achieved at 205 nm. Analysis of labeled compound was conducted using a Cd(Te) detector equipped with a RAD 501 single channel analyzer equipped HPLC.

Labeling with Tc-99m One milligram ascorbic acid was dissolved in 1 ml pure water, the pH adjusted to 5 with 0.1 N NH_3 solution, and then 200 μl of $SnCl_2 \cdot H_2O$ (1 mg/1 ml 0.1 N HCl) and 0.5 ml of $Na^{99m}TcO_4$ (74 MBq) in salina were added to the vial. The reaction vial was left at 25°C for 20 min. The radiochemical purity of the labeled compound was checked with RTLC, paper electrophoresis, and RHPLC. The labeling yield of ^{99m}Tc-AA was then determined to be 93±5.0%. The effect of pH on labeling yield was also investigated, and we examined labeling at pH 3, 5, and 7.

Stability of ^{99m}Tc-AA in Human Serum The stability of ^{99m}Tc-AA was examined by mixing 0.6 ml of the labeled compound and 1 ml of human serum. The sample was incubated at 37°C for 24 h. After 1, 2, 3, 4, and 24 h of incubation, the sample was analyzed. The amount of free pertechnetate in the sample was determined by RTLC using serum physiologic and ACD as eluent and the radioactivity was counted using a Cd(Te) detector.

***In Vivo* Studies** The Institutional Animal Review Committee of Ege University approved the animal experiments. ^{99m}Tc-AA was injected into the tail vein of male albino Wistar rats. The pH of ^{99m}Tc-AA, containing 300 μl (13.02 GBq/mmol) of ^{99m}Tc, was adjusted to 7 using 0.1 N NH_3. The solution was then passed through a Millipore filter (0.22 μm pore). The rats (155±11 g) were sacrificed by ether anaesthesia 15, 30, 60, and 120 min after-injection. Three rats were used for each time point. Their organs and blood were removed, weighed, and counted using a Cd(Te) detector equipped with a RAD 501 single channel analyzer. The percent injected dose per gram (%ID/g) for each organ and blood was then calculated.

Statistical Analyses Statistical analyses of the biodistribution results were performed using commercial software (SPSS 10.0 for Windows). After calculating the activity per gram for each organ, the relationship between the ^{99m}Tc-AA complex and organs was determined statistically by univariate analysis of variance. Statistical significance was taken as ($p<0.05$).

Results and Discussion

Radiochemical Purity According to the RTLC chromatograms, the *Rf* values of ^{99m}Tc-AA, $^{99m}TcO_4$-, and R-^{99m}Tc were 0.80—1.00, 0.72—1.00, and 0.60—0.88 respectively, in ACD eluent. The *Rf* values were 0.0—0.15, 0.68—0.80, and 0.0—0.13, respectively, in serum physiologic. ^{99m}Tc-AA has a negative charge according to paper electrophoresis experiments. In the RHPLC results, (Fig. 2) the R_t (retention time) value of ^{99m}Tc-AA was 1.04 min. The effect of pH on labeling yield was estimated. The labeling yields of ^{99m}Tc-AA were 81±3.0%, 93±5.0% and 74±6.0% at pH 3, 5 and 7, respectively. The stability experiment results in human serum showed that the labeling yield of ^{99m}Tc-AA decreased to 77.34±3.0% and 56.01±2.0% at the 4th and 24th h, respectively (Fig. 3). The effect of temperature (25 °C and 40 °C) on labeling yield was also investigated. The labeling yield of ^{99m}Tc-AA was 93±5.0% at 25 ºC and 53±3.0% at 40 °C. The labeling yield decreased due to increasing temperature because of the instability of AA at higher temperatures.[10]

***In Vivo* Experiments** The distribution of ^{99m}Tc activity in various tissues in the rats following a tail-vein injection of

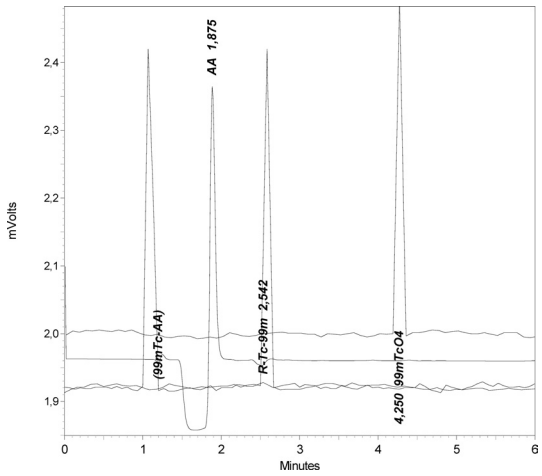

Fig. 2. HPLC (AA at UV Detector λ=205 nm) and RHPLC (^{99m}Tc-AA, $^{99m}TcO_4$-, R-^{99m}Tc)

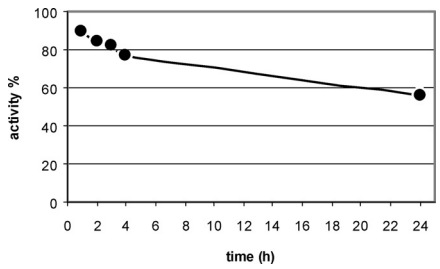

Fig. 3. Stability Curve of ^{99m}Tc-AA

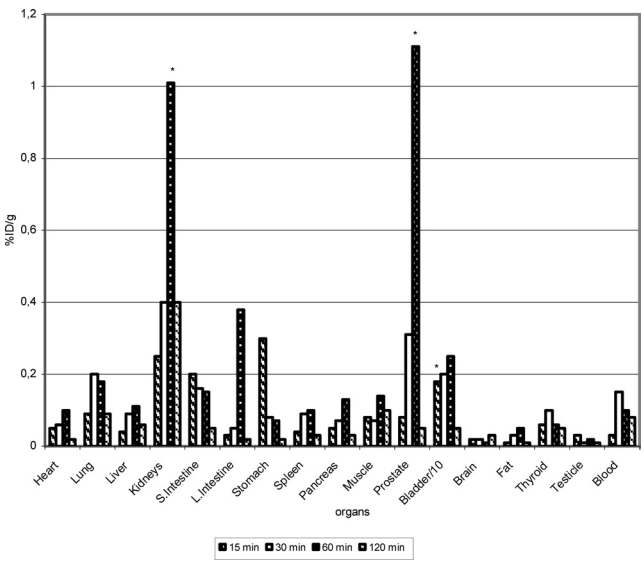

Fig. 4. Biodistribution of ^{99m}Tc-AA in Rats

* $p<0.05$.

^{99m}Tc-AA is shown in Fig. 4. The maximum uptakes of ^{99m}Tc-AA were observed in bladder, prostate, and kidneys at 60 min, in this order, after injection into albino Wistar rats (Fig. 4). While the %ID/g value of the labeled compound in the bladder, prostate and kidneys ($p<0.05$) was 2.5, 1.11 and 1.01 at 60 min, as shown in Fig. 4, the uptake by these organs had decreased at 120 min.

Discussion

In the present study, ascorbic acid was labeled with ^{99m}Tc with a high yield. The data obtained from *in vivo* experiments showed that ^{99m}Tc-AA is taken up well by the prostate and kidneys at 60 min. The labeled compound is cleared rapidly from the blood by the renal system. The concentration of AA in tissues varies widely in animal and human tissues. Tissues with the greatest levels of ascorbate are the adrenal and pituitary glands, followed by liver, spleen, eye lens, pancreas, kidneys, lungs, testicles, and brain in human, rats, and guinea pigs.[4,9,23,24] The results of many studies in animals using different amounts of ascorbate showed a similar trend. Yamamoto *et al.* conducted tissue distribution studies with 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid in rats and reported high uptake of the radioactivity in the adrenals, kidneys, liver, and small intestine which are organs known to have high concentrations of L-ascorbic acid. The uptake of radioactivity in the brain has been determined to be slow and low.[9] As can be seen in Fig. 4, the uptake of ^{99m}Tc-AA in brain is lower than other tissues. The highest amounts of [1-^{14}C-] labeled AA activity were found in liver, kidneys, and lung in rats by Hornig.[23] Although in our study low uptake of radioactivity in lung and liver compared to previous studies was observed, the data concerning the distribution of ^{99m}Tc-AA in brain and kidney in rats were in good agreement.

On the other hand, ascorbic acid labeled with ^{14}C was not retained over a longer period in liver, lung, and kidney after injection, suggesting that the organs have only a metabolizing or excretory function.[23] Our results indicated a longer retaining time for ^{99m}Tc-AA in kidneys and prostate, which might be due to the low clearance rates in these organs.

Conclusion

The maximum labeling yield of ^{99m}Tc-AA obtained was $93 \pm 5.0\%$ at pH 5 and 25 °C.

The radiopharmaceutical potential of ^{99m}Tc-AA in rats was investigated and it was found that ^{99m}Tc-AA uptake is high in the prostate and kidneys. For this reason, ^{99m}Tc-AA might be a good radiopharmaceutical for kidneys and prostate.

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References

1) Bijur G. N., Ariza M. E., Hitchcock C. L., Williams M. V., *Environ. Mol. Mutag.*, **30**, 339—345 (1997).
2) Fairfield K. M., Fletcher R. H., *JAMA*, **287**, 3116—3126 (2002).
3) Padh H., *Nutr. Rev.*, **49**, 65—70 (1991).
4) Rumsey S. C., Levine M., *Nutr. Biochem.*, **9**, 116—130 (1998).
5) Sauberlich H. E., *Annu. Rev. Nutr.*, **14**, 371—379 (1994).
6) Levine M., *N. Eng. J. Med.*, **314**, 892—902 (1986).
7) Levine M., Dhariwal K. R., Welch R. W., Yaohui W., Park J. B., *Am. J. Clin. Nutr.*, **62**, 1347S—1366S (1995).
8) Levine M., Rumsey S. C., Daruwala R., Park J. B., Wang Y., *JAMA*, **281**, 1415—1423 (1999).
9) Yamamoto F., Sasaki S., Maeda M., *Appl. Radiat. Isot.*, **43**, 633—639 (1992).
10) Ments N. K., *Haper'in Biyokimyaya Bakıs.*, **1998**, 838—840 (1988).
11) Jacob R. A., Sotoudeh G., *Nutr. Clin. Care*, **5**, 66—74 (2002).
12) DC Nutrition, "General discussion of vitamins" ⟨http://dcnutrition.com/vitamins/⟩.
13) Vera J. C., Rivas C. I., Vesasquez F. V., Zhang R. H., Concha I. I., Golde W. D., *J. Biol. Chem.*, **40**, 23706—23712 (1995).
14) Angulo C., Rauch M. C., Droppelmann A., Reyes A. M., Slebe J. C., Lopez F. D., Guaiquil V. H., Vera J. C., Concha I. I., *J. Cell Biochem.*, **71**, 189—203 (1998).
15) Krishnaja A. P., Sharma N. K., *Teratog. Carcinog. Mutag.*, **1** (Suppl.), 99—112 (2003).
16) Agus D. B., Gambhir S. S., Pardridge M., Spielholz C., Baselga J., Vera J. C., Golde D. W., *J. Clin. Invest.*, **100**, 2842—2848 (1997).
17) Baker E., Hodges R. E., Hood J., Sauberlich H. E., March S. C., *Am. J. Clin. Nutr.*, **22**, 549—558 (1969).
18) Tolbert B., Chen A. W., Bell E. M., Baker E. M., *Am. J. Clin. Nutr.*, **20**, 250—252 (1967).
19) Tsukaguchi H., Tokui T., Mackenzie B., Berger U. V., Chen X. Z., Wang Y., Brubaker R. F., Hediger M. A., *Nature* (London), **399**, 70—72 (1999).
20) Wright J. R., Castranova V., Colby H. D., Miles P. R., *J. Appl. Physiol.*, **5**, 1477—1483 (1981).
21) Fiorani M., Sanctis R. De., Scarlatti F., Vallorani L., Bellis R. D. E., Serafini G., Bianchi M., Stocchi V., *Mol. Cell Biochem.*, **209**, 145—153 (2000).
22) Wu H. C., Lu H. F., Hung C. F., Chung J. G., *Urol. Res.*, **28**, 235—240 (2000).
23) Hornig D., *Ann. N.Y. Acad. Sci.*, **258**, 103—118 (1975).
24) Subcommittee on Vitamin Tolerance, Committee on Animal Nutrition, Board on Agriculture (BOA), National Research Council, U.S.A., "Vitamin Tolerance of Animals," National Academic Press, Washington D.C., 1987, pp. 36—42 ⟨http://www.nap.edu/books/030903728X/html/36.html⟩.