International Journal of Molecular Medicine Advance Sciences, 1 (2): 177-181, 2005

Assessment of the Effect of an Extract of Chitosan on the Labeling of Blood Elements with Technetium-99 M: An *in vitro* Study

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Abstract: Human beings have been widely used natural products as medicines. However, there are many reports about their undesirable toxicological effects. Chitosan is a kind of polysaccharide derived from the chitin (copolymer of α-(1→4)-D-glucosamine and β-(1→4)-N-acetil-D-glucosamine) that it is found in abundance in the nature, mainly crustaceans. It Had to the basic character, attributed to the presence of the grouping amine in the repeated units, and to its biodegradability, these two polymers come sufficiently interesting in scientists and technologists, who have discovered diverse applications, especially in the biomedical area. It is concerned that many natural remedies may contain potentially toxic ingredients and contaminants such as heavy metals. Red Blood Cells (RBC) and plasma proteins labeled with technetium-99 m (99 mTc) have several clinical applications and it has been reported that some natural products are capable of reducing the efficiency of this radiolabeling. The aim of this study was to assess the effect of a chitosan extract on the labeling of blood elements with 99 mTc. In the preparation of the extracts it was used 310mg of chitosan diluted in 10mL of saline solution (NaCl 0.9%). Samples (0.5 mL) of blood from Wistar rats were incubated with 0.1 mL of the extracts during 1 hour. After that, the samples were incubated with stannous chloride ($SnCl_2$) and 99 mTc. The blood was centrifuged and Plasma (P) and RBC were isolated. P and RBC were also precipitated with Trichloroacetic acid (TCA) 5% and Soluble (S) and Insoluble (I) Fraction (F) was determined. The results have shown that the extract has not altered the radiolabeling . It was described that some extracts as *Fucus vesiculosus, Paullinia cupana, Mentha crispa* L were able to alter the radiolabeling of blood elements. In the light of the results obtained we suggest that the referred extract has an antioxidant properties.

Key words: Chitosan, red blood cells, plasma proteins and technetium-99 m

INTRODUCTION

Natural products are widely used as food or food additives or as an in folk medicine as an alternative way of treatment by humans. Phytoremedy are widely used in folk medicine for human beings and the sale of these plants has increased considerably over the last 10 years in the industrialized countries. Aqueous extracts of many natural products are widely used in therapy as complementary medicines[1,2].

Traditional Chinese Herbal Medicines (TCHM) are increasingly used throughout the Earth, as they are considered to be effective and to have few side effects. Contaminants of TCHM include heavy metals and undeclared drugs[3]]. Biological effects of metals have been reported as a effect of the transition metals which catalyzefree radical production that can be related to aging processes and neurodegeneretive diseases such as Alzheimer's disease, Parkinson's disease[4].

Chitosan is a kind ok polysaccharide derived from the chitin (copolymer of α-(1→4)-D-glucosamine and β-(1→4)-N-acetil-D-glucosamine), that it is found in abundance in the nature, mainly crustaceans. It had to the basic character, attributed to the presence of the grouping

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amine in the repeated units, and to its biodegradability, these two polymers come sufficiently interesting in scientists and technologists, who have discovered diverse applications, especially in the biomedical area[5]. The physico-chemical properties of chitosan dependent on the average degree of acetylation and of the average molar mass. Several applications of chitosan have been proposed in the literature, mainly in water treatment, cosmetic and drug manufacturing, food additives, semi-permeable membranes and development of biomaterials[6].

The biodistribution of the radiopharmaceuticals can be altered by natural and synthetic drugs as well as the radiolabeling of blood elements with technetium-99 m (^{99m}Tc)[7-9]. When a radionuclide have its capability to bind to blood elements altered by drugs therapy, the process of labeled red blood cells may be repeated, resulting in an additional radiation dose to the patient[10,11].

^{99} ^{m}Tc has been the most utilized radionuclide in nuclear medicine procedures and it has also been used in basic research[12,13]. The wide utilized in nuclear medicine is due to its optimal physical characteristics (half-life of 6h, gamma rays energy of 140 KeV and minimal dose to the patients), convenient availability from 99 mo/^{99} ^{m}Tc generator and negligible environmental impact. Nearly almost all scanning devices currently in use are optimized for detecting the electromagnetic emission from this radionuclide[10,11].

There are many applications of ^{99} ^{m}Tc-labeled Red Blood Cells (RBC). The most important is in cardiovascular nuclear medicine, where one tries to image the heart to determine its functional status as a pump, to calculate the left ventricular function by measuring the ejection fractions, and to evaluate wall motion abnormalities. Some other applications are in the detection of gastrointestinal bleeding, and in the determination of the RBC mass in patients[10,11].

The labeled process with ^{99} ^{m}Tc depends on a reducing agent and stannous ion (Sn^{2+}), mainly as stannous chloride, is usually used for this purpose[13,14]. When whole blood is used in the labeling of RBC with ^{99} ^{m}Tc, radioactivity is found on blood cells, however it is also bound on plasma proteins. This labeling process depends on optimal stannous chloride concentration and stannous and pertechnetate ions across the RBC membrane, probably spending energy and the radionuclide is mainly bound to hemoglobin molecule[10,15]. Several of the cellular labeling steps have been well characterized. The band-3 anion transport system and calcium channels may be the ways that ^{99} ^{m}Tc and Sn^{+2}, respectively, reach the interior of the RBC. If one damages the RBC, one can do selective spleen imaging since the spleen rapidly sequestrates damaged cells. RBC have been labeled with ^{99} ^{m}Tc for *in vitro in vivo* or *in vivo/ in vitro* techniques[16].

Plasma proteins (PP) have also been labeled with the referred radionuclide. ^{99} ^{m}Tc -labeled PP has been used to locate placenta, to evaluate the cardiac function and pulmonary perfusion, to determine blood volume and to study the gastrointestinal protein loss[10,11].

The labeling of red blood cells with ^{99} ^{m}Tc has been influenced by patient medications, by the labeling conditions [10,11,17] or by the presence of extracts of plants, as *Paullinia cupana*[18], *Maytenus ilicifolia*[19], *Thuya occidentalis*[8], *Nicotiana tabacum*[9], and. Nevertheless, there is not a well established *in vitro/in vivo* model to study the interaction of therapeutic drugs with radiopharmaceuticals[10,14,17]]. Then, we have evaluated the influence of a Chitosan extract (i) on the labeling of blood elements with ^{99} ^{m}Tc.

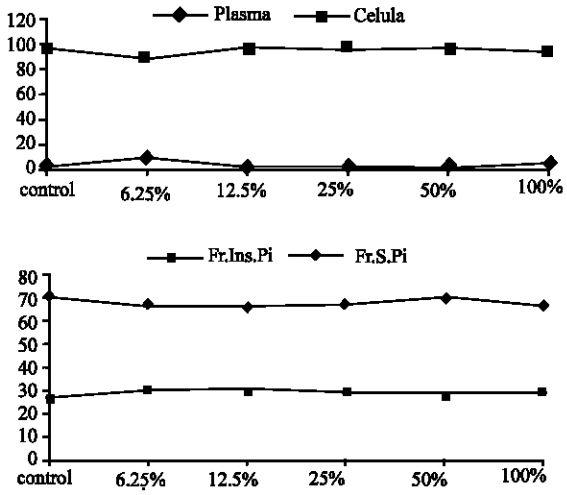
MATERIAL AND METHODS

In this experimental it was used the extract bought of Herbarium Laboratory [Quitosana Lot: 228103 (produced October, 2003 and validity October, 2005). To prepare the solution which was considered like 100% it was diluted 310 mg of chitosan into 10ml of saline solution (NaCl 0.9%) obtained a solution 100% (31mg mL. The extract was diluted (50%) in different concentrations (50; 25 12.5 and 6,25%v/v).

Samples of 0.5 mL of blood were withdrawn from Wistar rats and incubated with 0.1 mL of the extract during 1 h. In the control group saline solution was used. Elapsed this period of time it was added 0.5 mL of stannous chloride (1.2 μg mL^{-1}), as $SnCl_2.2H_2O$ for 1 hour at room temperature. After this period of time, 99 mTc (0.1 mL), as sodium pertechnetate, was added and the incubation continued for another 10 min. These samples were centrifuged and Plasma (P) and Blood Cells (BC) were separated. Samples (20 μL) of P and BC were precipitated with 1 mL of trichloroacetic acid (TCA) 5% and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in P, BC, IF-P, SF-P, IF-BC and SF-BC were determined in a well counter. After that, the % of radioactivity (%ATI) was calculated, as previously reported [20]. A statistical analysis (Tukey-Kramer Multiple Comparisons Test, n=5) was utilized to compare the experimental data.

RESULTS

Figure1 shows the effect of chitosan extract on the labeling of blood elements with 99 mTc. The analysis



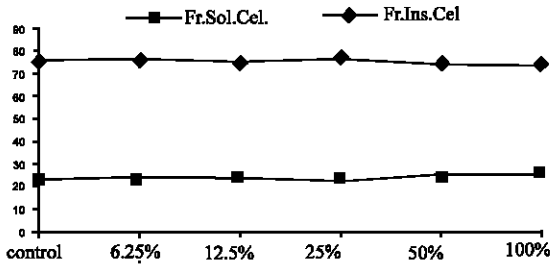
of the results revealed that there was not an alteration on the labeling of red blood cells (BC) extract.

Samples of heparinized blood from *Wistar* Rats were incubated for 1 hour with stannous chloride (1.2 μg mL^{-1}) and 99 mTc, as sodium pertechnetate were added. These samples were centrifuged and Plasma (P) and Blood Cells (BC) were separated. Samples (20 μL) of BC were precipitated with Trichloroacetic Acid (TCA) 5% and Soluble (SF) and Insoluble Fractions (IF) were separated. The radioactivity in IF-P and SF-P was determined in a well counter and the % of radioactivity (% ATI) was calculated. A statistical analysis (Tukey-Kramer Multiple Comparisons Test, n=5) was used to compare the results. The values are averages.

Figure 2 shows the effect of chitosan extract on the labeling of blood elements with 99 mTc. The analysis of the results revealed that there was not an alteration on the efficiency of radiolabeling of the insoluble fraction of plasma (IF-P) in the presence of the extracts.

Samples of heparinized blood from *Wistar* Rats were incubated for 1 hour with stannous chloride (1.2 μg mL^{-1}) and 99 mTc, as sodium pertechnetate were added. These samples were centrifuged and Plasma (P) and Blood Cells (BC) were separated. Samples (20 μL) of BC were precipitated with trichloroacetic acid (TCA) 5% and soluble (SF) and Insoluble Fractions (IF) were separated. The radioactivity in IF-P and SF-P was determined in a well counter and the % of radioactivity (% ATI) was calculated. A statistical analysis (Tukey-Kramer Multiple Comparisons Test, n=5) was used to compare the results. The values are averages.

Figure 3 shows the effect of chitosan extract on the labeling of blood elements with 99 mTc. The analysis of the results revealed that there was not an alteration on the efficiency of radiolabeling of the Insoluble Fraction of Cell (IF-C) in the presence of the extracts.



Samples of heparinized blood from Wistar Rats (treated or not with the extracts) were incubated for 1 hour with stannous chloride (1.2 μg mL^{-1}) and 99 mTc, as sodium pertechnetate were added. These samples were centrifuged and Plasma (P) and Blood Cells (bc) were separated. Samples (20 μL) of BC were precipitated with Trichloroacetic Acid (TCA) 5% and Soluble (SF) and Insoluble Fractions (IF) were separated. The radioactivity in IF-P and SF-P was determined in a well counter and the % of radioactivity (% ATI) was calculated. A statistical analysis (Tukey-Kramer Multiple Comparisons Test, n=5) was used to compare the results. The values are averages.

DISCUSSION

Many authors have described a great number of drugs, which can be due to the causes of some diseases of red cells. There are evidences that drugs can affect either radiolabeling or biodistribution of blood cells in the context of the nuclear medicine. In the literature some researches have turned their attention to *in vitro* testing of the drug with labeled cells[14,20, 21]. We agree with Hesselewood and Leung[11], that many reports on drug interactions with radiopharmaceuticals are anecdotal and in some instances a direct cause and effect relationship has not been unequivocally established. This fact could be diminished with the development of *in vitro* tests to evaluate the drug/radiopharmaceuticals interactions and the consequence for the bioavailability of the radiopharmaceuticals and the labeling of blood constituents. There are concerns that some natural medicines may contain potentially toxic ingredients and contaminants such heavy metals[3]. Some substances may alter the labeling of blood constituents with 99 mTc[2]. Previous studies have demonstrated that natural extracts may alter the labeling of blood elements with ^{99m}Tc[21].

In the labeling process of blood constituents with 99 mTc is needed a reducing agent, and probably the stannous ion would be oxidized. In *in vitro* studies was verified that the extracts of *Thuya ocidentallis*[8], *Nicotiana tabacum*[9], *Maytenus ilicifolia*[19], *Syzygium jambolanum*, *Stryphnodendron adstringens* (Mart). *Coville*[19] and *Ginkgo biloba* possibly, would have

oxidants compounds, and the labeling of blood elements decrease in the presence of these extracts. The use of natural products, as medicinal plants, is very frequent in folk medicine around the world and chitosan is utilized of several applications, which have been proposed in the literature, mainly in water treatment, cosmetic and drug manufacturing, food additives, semipermeable membranes and development of biomaterials[6].

In the present study, chitosan extract was not capable to alter the radiolabeling of blood constituents with ^{99m}Tc like it was observed with an extract of cauliflower (leaf)[22]. In other study, Diré et al[7], in a *in vitro* study eyed that the chayotte extracts were not capable of altering the radiolabeling of blood constituents different from the results obtained in an *in vivo* study with the referred extract once it was observed that the extract of chayotte when ingested by Wistar rats has promoted alteration on the efficiency on the radiolabeling of sanguineous elements.

Many reports about medicine plants are rarely written up in the traditional medicine literature. In order to make an accurate assessment of the impact of drugs and other factors on the biological systems additional data are required[14,20,21].

CONCLUSIONS

It can suggest that the effect of chitosan could be explained by its antioxidant properties, which can be due to the fact that chitosan may be heavily metabolized by the liver, resulting in inactive metabolites that do not alter the labeling of blood elements with ^{99m}Tc.

ACKNOWLEDGEMENTS

This study supported by Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Universidade do Estado do Rio de Janeiro (UERJ)

REFERENCES

1. Capasso, R., A.A. Izzo, L. Pinto, T. Bifulco, C. Vitobello, N. Mascolo, 2000. Phytotherapy and quality of herbal medicines. Fitoterapia., 71: 58-65.
2. Oliveira, J. F., M. B. Oliveira, A. S. Ávila, A. C. S. Braga, M. T. J. A. Catanho, R. L. C. Jales, V. N. Cardoso and M. Bernardo-Filho, 2003. Assessment of the effect of *Fucus vesiculosos* extract on the labeling of. blood constituents with technetium-99 m and the histological modifications on the shape of the red blood cells. Food Chem. Toxicol., 41: 15-20
3. Kam, P. C. A. and S. Liew, 2002. Traditional Chinese herbal medicine and anaesthesia. Anaesthesia, 57: 1083-1089.
4. Silva, C.R., M.B.N. Oliveira, S.F. Melo, F.J.S. Dantas, J.C.P. Mattos, R.J.A.C. Bezerra, A. Caldeira-de-Araújo, A. Duatti, M. Bernardo-Filho, 2002. Biological effects of stannous chloride, a substance that can produce stimulation or depression of the central nervous system. Brain Res. Bull., 59: 213-216.
5. Canella, Mª. Kathya, Nilza de Carvalho and Garcia, Rosangela Balaban, 2001. Characterization of chitosan by Gel Permeation Chromatography - Influence of preparation method and solvent. Quím.. Nova., 24: 13-17.
6. Santos, José E. dos, João da P. Soares, Dockal and R. Edward *et al*., 2003. Characterization of commercial chitosan from different suppliers. Polímeros., 13: 242-249.
7. Diré, G., E. Lima, D. Mattos, M.B. Oliveira, M.J. Pereira, Moreno, R. Freitas, M.L. Gomes and M. Bernardo-Filho, 2001. J. Labeled Compds. Radiopharm., 1: 44.
8. Oliveira, J.F., A.C.S. Braga, A.S.R. Ávila, B Gutfilen and M. Bernardo-Filho, 1997. Effect of *Thuya occidentalis* on the labeling of red blood cells and plasma proteins with technetium-99 m. Yale J. Biol. Med.1997; 69: 489.
9. Vidal, M.V., B. Gutfilen, L.M. Barbosa-da-Fonseca and M. Bernardo-Filho, 1998. J. Exp. Clin. Cancer Res., 17: 41.
10. Early, P.J. and D.B. Sodee, 1995. Principles and Practice of Nuclear Medicine. Mosby-Year Book, Inc., Toronto., 877.
11. Hesselewwod, S. and E. Leung, 1994.Drug interactions with radiopharmaceuticals. Eur. J. Nucl. Med., 21: 348.
12. Mattos, D.M.M., M.L. Gomes, R.S. Freitas, S. Moreno, G.L. Lima-Filho, E.F. Paula, R.L.C. Jales, Bernardo-Filho, 2001. Which are the most used radionuclides in the pet and in the spect techniques in the world. J. Labeled Cpd. Radiopharm., 1: 44.
13. Saha, G.B., 1998. Fundamentals of Nuclear Pharmacy. Springer-Verlag, New York, pp: 331.
14. Sampson, C.B., 1996. Nucl. Med. Commun., 17: 648.
15. Gutfilen, B. L. and F.S. Pontes, I.S.C. Alencar and M. Bernardo-Filho, 1993. Biomed. Letter, 48: 305.
16. Srivastava, S.C., R.F. Straub and P. J. Richards, 1984. Labeled Compds. Radiopharm., 21: 1055.
17. Santos, J.S., E.F. Paula, T.G. Correa , L.C. Freitas, L.M. Fonseca, B. Gutfilen and M. Bernardo-Filho, 1995. Braz. J. Med. Biol. Res., 41: 1575.

18. Oliveira, J.F. A.S. Ávila, A.C.S. Braga, M.B.N. Oliveira, E.M. Boasquevisque, R.L. Jales, V.N. Cardoso and M. Bernardo-Filho, 2002. Effect of extract of medicinal plants on the labeling of blood elements with Technetium-99 m and on the morphology of red blood cells: A study with *Paullinia cupana. Fitoterapia*., 73: 305.
19. Oliveira, J.F., A.C.S. Braga, A.S.R. Ávila, A.C. Araújo, V.N. Cardoso, R.J.A.C. Bezerra and M. Bernardo-Filho, 2000. Assessment of the effect of Maytenus ilicifolia (espineira santa) extract on the labeling of red blood cells and plasma proteins with technetium-99 m. J. Ethnopharmacol., 72: 179.
20. Bernardo Filho, M., B. Gutfilen and O.S. Maciel, 1994. Effect of different anticoagulants on the labelling of red blood cells and plasma proteins with Tc-99 m. Nucl. Med. Comm.., 15:730.
21. Braga, A.C.S., M.B.N. Oliveira, G.D. Feliciano, I.W. Reninger, J.F. Fonseca,C.R. Silva and M. Bernardo-Filho, 2000. The effect of drugs on the labeling of blood elements with technetium-99 m. Curr. Pharm. Design., 6: 1179.
22. Lima, E.A.C., G. Diré, D.M.M. Mattos, R. Freitas, M.L. Gomes, M.B. Oliveira, M.V.C. Faria, R.L. Jales and M. Bernardo-Filho, 2002. Effect of an extract of cauliflower (leaf) on the labeling of blood elements with technetium-99 m and on the survival of Escherichia coli AB1157 submitted to the treatment with stannous chloride. Food Chem. Toxicol., 40: 919-923.