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The Analysis of the Effect of a Chayotte Extract on the Radiolabeling of Blood Elements in Diabetic Rats

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Abstract: The use of natural products occurs around the world. The labeling of blood constituents with technetium-99m (99m Tc) has been influenced by natural extracts and oxidative stress. Some studies suggested that maternal diabetes can affect the embryology environmental and this fact could help to elucidate that the oxidative stress may be related to the disturb of the gene expression which is essential in the control of the ontogenetic processes. We evaluated the influence of a chayotte(*Sechium edule*) extract on the labeling of blood elements with 99m Tc in diabetic female rats. The animals were treated with chayotte during 7 days and samples of blood were withdrawn. The samples were incubated with stannous chloride and with 99mTc. Plasma(P) and blood cells (BC) were isolated, also precipitated with trichloroacetic acid and soluble (SF) and insoluble fractions (IF) separated. The radioactivity (ATI%) was rated in RBC, IF-P and IF-C in a well counter. In the diabetic group it was observed an increase in the radioactivity in BC (from 43.65 ± 1.83 to 59.47 ± 1.83 and in the IF-BC (from 26.22 ± 23.58 to 73.28 ± 23.58). It was noticed that the referred extract has normalizing the efficiency of radiolabeling in the diabetic animals which have received the referred extract. The effect of chayotte extract probably, could be explained by the metabolization of the chayotte that would be capable of inducing the generation of active metabolites with oxidant properties probably altering the activity of cell membrane.

Key words: Chayotte, technetium-99m, diabetes, blood elements

Introduction

It has been reported that natural and synthetic drugs are able to alter the biodistribution of different radiopharmaceuticals (Hesslewood and Leung, 1994; Early and Sodee, 1995). *Sechium edule* (chayotte), a subtropical vegetable with potent diuretic action, is a cucurbitaceus specie which is used as food or as medication in popular medicine. It was reported a case of severe hypokalemia pregnancy and that a chayotte preparation was implicated, as the potassium level returned to normal, without recurrence of hypokalemia, once the ingestion of this vegetable was stopped (Jensen and Lai, 1986; Flores, 1989). Technetium-99m (99mTc) has been the most utilized radionuclide in nuclear medicine procedures (Oliveira *et al*., 1997) and it has also been used in basic research (Gutfilen *et al*., 1996; Gordon, 2000). The wide use 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 99Mo/99mTc generator and negligible environmental impact). Nearly almost all scanning devices currently in use are optimized for detecting the eletromagnetic emission from this radionuclide (Saha, 1998).

It is known many applications of 99mTc-labeled red blood cells (99mTc-RBC), as in cardiovascular evaluations, in the detection of gastrointestinal bleeding and in the determination of the RBC mass in patients. RBC have been labeled with 99mTc through of *in vitro, in vivo* or *in vivo*/*in vitro* techniques (Srivastava *et al*., 1992; Early and Sodee, 1995). In spite of that, there is not a well established model to evaluate the effects of drugs (synthetic or natural) on the radiolabeling of blood components. According to Roush (1996) it is suggested that oxidative stress may be resulted by the exposition to some drugs, ionizing radiation and deficiency of folic acid. Insulin resistance, characterized by an inexorable decline in skeletal muscle glucose utilization and/or an

excessive hepatic glucose production, constitutes a major pathogenic importance in a cluster of clinical disorders including diabetes mellitus, hypertension, dyslipidemia, central obesity and coronary artery disease. A novel concept suggests that heightened state of oxidative stress during diabetes contributes, at least in part, to the development of insulin resistance (Bitar *et al*., 2005).

According to Love and Oldford (2005) cardiovascular disease (CVD) and diabetes are growing public health burdens and remain one of the leading causes of morbidity and mortality in Canada (Heart and Stroke Foundation, 2003). It has become increasingly evident that individuals who present with a cluster of metabolic disorders, known as the metabolic syndrome, are at an increased risk of developing both CVD and type 2 diabetes. Some studies suggested that maternal diabetes can affect the embryology environmental and this fact could help to elucidate that the oxidative stress may be related to the disturb of the gene expression which is essential in the control of the ontogenetic processes. Bruce (2003) has described that aging is accompanied by decreased specific activity in many enzymes, altered heat stability, and increased carbonyl content of proteins. The noenzymatic reaction of carbohydrates with amino groups of proteins (glycation) can give rise to advanced glycation end-products (AGEs). These AGEs increase with aging and are implicated in diabetes, eye disorders, and amyloid accumulation. Many extracellular matrix proteins exhibit increased cross-linking with age. The characterization of moleculary defined AGEs, particularly those of potential pathophysiological relevance, remains a challenging area of investigation. The most important work in this area continues to focus on the structural analysis of cross-linking moieties derived from Maillard reaction (Bucala, 2002).

Recently, a significant new fraction of. total AGEs, with relevant effects not only on protein structure and function, but also as mediators of biological responses, have been characterized in tissues (Basta *et al*., 2002). In this study, we have evaluated the influence of a chayotte extract and diabetic on the labeling of blood constituents with 99mTc using an *in vitro* technique.

Materials and Methods

Preparing and analysis of the extract: Chayotte was purchased from a local market in Rio de Janeiro city, RJ, Brazil. To prepare the extract, 50 g of skin of chayotte were mixed with 500 mL of water in an electric extractor. This preparation was filtered and this extract was considered 100%.

The presence of toxic compounds was evaluated and we did not find them in the extracts of chayotte used in our experiments. The method to verify the presence of these toxic products is based on inhibition of acetylcholinesterase in the presence of the pesticides (Cunha Bastos *et al*., 1991). In this method, brain acethylcholinestarase is utilized as an *in vitro* detector of organophosphorus and carbamate insectides. Briefly, a preparation of acetylcholinesterase was obtained after extraction of a rat brain microsomal fraction with Triton X-100 and was incubated with the extract of chayotte. Enzyme assay was performed by a potentiometric method based on the formation of acetic acid in the incubation mixture (preparation of acetylcholinesterase and extract of chayotte)

Diabetes induction: The injection of Streptozotocine was realized in the ventral region next to the alba line with a unique dose of 30 µg/kg by body weight dissolved in saline solution or in a same volume of citrate (control group). In a period of 2h after the injection the rats were maintained without water and after that it was added sugar high quantities in their drinking during 1.5h. After 48h of the induction it was performed the rate of sugar tests by tail puction. It was considered diabetic the rats with rate of sugar rates above 180 dg/dL.

Bioavailability experimental: The experiments were performed with the chayotte extract administrated to the animals. The vegetable extract was prepared in the concentration of 0.1 $g.mL^{-1}$ and it was used the skin of the chayotte. The animals have been divided into 4 groups (control, diabetic, treated with chayotte and diabetic treated with the chayotte extract), each group with 4 animals. The extract has been administrated to the animals during 7 days. After this period of time, 99mTc (0.3 mL), as sodium pertechnetate, was injected by ocular plexus and the after 10 min samples of blood were withdraw. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 µL) of P and BC were also 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. Statistical analysis (Dunnett test) was performed to compare the experimental data.

Results

Due to the analysis of the results it was noticed that there was an increase in the radioactivity in BC (from 43.65 ± 1.83 to 59.47 ± 1.83 and in the IF-BC (from 26.22 ± 23.58 to 73.28 ± 23.58) in the diabetic group although in the diabetic group which has been treated with chayotte extract it was not observed an altering in the radiolabeling of blood elements.

The animal have been treated with chayotte extract during 7 days. After this period of time, 99mTc (0.3 mL), as sodium pertechnetate, was injected by ocular plexus

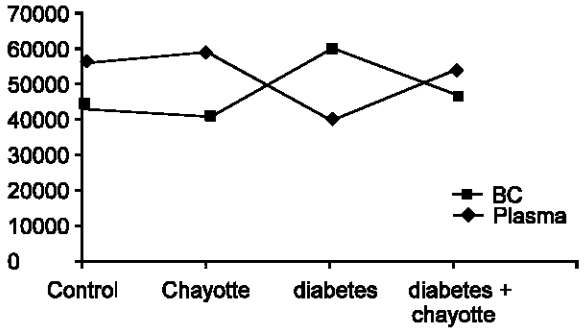

Fig. 1: Effect of chayotte extract and diabetes on the radiolabeling of blood cells

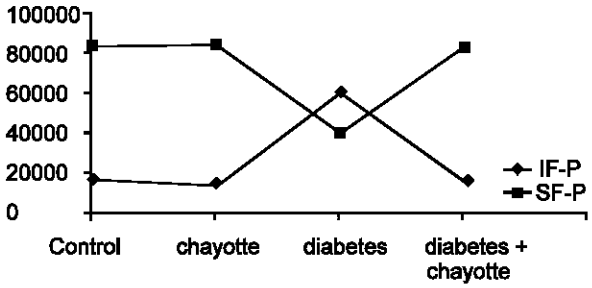

Fig. 2: Effect of chayotte extract and diabetes on the radiolabeling of Plasma Proteins

and the after 10 min samples of blood were withdraw. These samples were centrifuged and P and BC were separated. Samples (20 µL) of P and BC were diluted in 1 mL of distilled water. The radioactivity in P and BC were determined in a well counter. After that, the %ATI was calculated. Statistical analysis (Dunnett test) was performed to compare the experimental data.

The animal have been treated with chayotte extract during 7 days. After this period of time, 99mTc (0.3 mL), as sodium pertechnetate, was injected by ocular plexus and the after 10 min samples of blood were withdraw. These samples were centrifuged and P and BC were separated. Samples (20 µL) of P were also precipitated with 1 mL of TCA 5% and SF and IF were separated. The radioactivity in IF-P and SF-P were determined in a well counter. After that, the %ATI was calculated. Statistical analysis (Dunnett test) was performed to compare the experimental data.

The animal have been treated with chayotte extract during 7 days. After this period of time, 99mTc (0.3 mL), as sodium pertechnetate, was injected by ocular plexus and the after 10 min samples of blood were withdraw. These samples were centrifuged and P and BC were separated. Samples (20 µL) of BC were also precipitated with 1 mL of TCA 5% and SF and IF were separated. The radioactivity in IF-BC and SF-BC were determined in a well counter. After that, the %ATI was calculated. Statistical analysis (Dunnett test) was performed to compare the experimental data.

Discussion

A therapeutic drug is capable to modify the nature/amount of the 99mTc-radiopharmaceutical bound to the blood elements and this may result in unexpected behavior of the radiopharmaceutical. Therapeutic drugs and extracts of medicinal can also alter the labeling of blood elements with technetium-99m (Sampson, 1996; Reiniger *et al.*, 1999). We agree with Hesslewood and Leung 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 (Srivastava *et al.*, 1992).

In an *in vivo* study Diré *et al.*, 2001 described that chayotte extracts (macerated and decoct) were capable of altering the labeling of blood elements with 99mTc. Lima *et al.*, 2001 described that a leaf extract isolated from cauliflower which was administrated to the animals during the same time was not capable to alter the radiolabeling of blood elements. In the labeling process of blood elements with 99mTc needs a reducing agent, and probably the stannous ion would be oxidized. In *in vitro* studies was verified that extracts of *Thuya ocidentallis* (Oliveira *et al.*, 1997), *Nicotiana tabacum* (Braga *et al.*, 2000) and *Maytenus ilicifolia* (Oliveira *et al.*, 2000), possibly, would have oxidants compounds, and the labeling of blood elements decrease in the presence of these extracts. It was reported that *Sechium edule* extract was capable to alter the biodistribution of 99m-Tc-radiopharmaceutical (Diré *et al.*, 2001).

Many properties are attributed to the chayotte one of them is the hypotensor effect which was observed by Gordon *et al.*, 2000. This effect could be due to the action of metabolits which were produced by the metabolism of chayotte. The diuretic effect described by Jensen *et al.*, 1986 may support the action of chayotte described by Gordon. In this study we observed an increase in the radiolabeling of blood cells and in the insoluble fraction of the blood cells in the animals treated with the referred extract in diabetic rats in comparison to the diabetics animals treated with chayotte extract.

The genotoxic effect of *Paullinia cupana* (Fonseca *et al.*, 1995) and *Brassica oleracea* (cauliflower) (Lima *et al.*, 2002), a natural products, could be associated to the generation of reactive oxygen species (ROS) that are oxidant agents. Sohal and Weindruch (1996) have described that oxygen-derived species can react with macromolecules in a self-perpetuating manner; they create free radicals out of subsequently attacked molecules, which in turn create free radicals out of other molecules, thereby amplifying the effect of the initial free

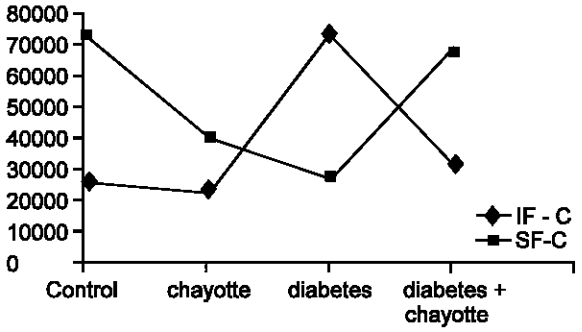

Fig. 3: Effect of chayotte extract and diabetes on the radiolabeling of Cell Proteins

radical attack. Reactive oxygen species appear to play a role in regulating differential gene expression. It is know that in diabetic is observed an decrease in the pH of blood (Kierszenbaum, 2004). In this study we can speculate that this fact could be associated with the increase on the labeling of blood cell and cell proteins with 99mTc due to the fact that blood cells may participate in the control of blood pH. Alterations on the shape of the red blood cells were found with blood treated with tobacco (Braga *et al.*, 2000), *Sechium edule* (Diré *et al.*, 2001) and *Maytenus ilicifolia* (Oliveira *et al.*, 2000). These studies may support an action of the extract in the cell membrane. Related to Bucala (2002) it was verified that in diabetes has an increased AGE formation and high circulating levels of glycated hemoglobins, including two diagnostically useful species, the hemoglobin Amadori product and the hemoglobin AGE product. This fact may support an increase of the %ATI linked to blood proteins due to the purpose that the quantification of AGE-modified forms of human hemoglobin and low-density lipoprotein.

There is not a well established model to study the interaction of therapeutic drugs (natural or synthetic) with radiopharmaceuticals. However, care must be taken when attempting for extrapolate experimental data to the clinical situation, once the observed effects may depend on the amounts of the drug (Santos *et al.*, 1995).

Conclusion: In general, we can suggest that *Sechium edule* extract is capable of maintaining the efficiency of labeling of blood elements with 99mTc although diabetic status alter the radiolabeling of blood constituents. In this case, we suggest that these effects can be due to the generation of active metabolites with oxidant properties which may altering the function and structures of proteins.

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