Effect of a Chayote (Sechium edule) extract on the radiolabelling of blood elements in diabetic rats.

Efecto del extracto de Chayote (Sechium edule) en el radiomarcado de elementos hemáticos en ratas diabéticas.

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Abstract

The labeling of blood constituents with technetium-99m (99mTc) has been influenced by natural extracts and oxidative stress. Some studies suggested that maternal diabetes can affect the embryo environment and this fact could help to elucidate if the oxidative stress may be related to the disturbance of the gene expression which is essential in the control of the ontogenetic processes. We evaluated the influence of a chayote (Sechium edule) extract on the labeling of blood elements with 99mTc in diabetic female rats. The animals were treated with chayote during 7 days and samples of blood were withdrawn.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, precipitated with acido tricloroaético, obtaining the fractions soluble (SF) e insoluble (IF). The radioactivity (ATI%) was rated in BC, 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 chayote extract probably could be explained by the metabolization of the chayote that would be capable of inducing the generation of active metabolites with oxidant properties probably altering the activity of cell membrane.

Palabras clave: chayote, tecnecio-99m, diabetes, elementos hemáticos.
Introduction

It has been reported that natural and synthetic drugs are able to alter the biodistribution of different radiopharmaceuticals (1, 2). Chayote (Sechium edule, Cucurbitaceae) is a subtropical vegetable with potent diuretic action, which is used as food or as medication in popular medicine. It has been reported a case of severe hypokalemia during the pregnancy period, and that a chayote preparation was implied, considering the potassium level returned to normal, without recurrence of hypokalemia, once the ingestion of this vegetable was stopped (3, 4). Technetium-99m (99mTc) has been the most used radionuclide in nuclear medicine procedures (5) and it has also been used in basic research (6, 7). The extensive 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). Almost all scanning devices currently in use are optimized for detecting the electromagnetic emission from this radionuclide (8).

It is known that many applications of 99mTc-labeled red blood cells (99mTc-RBC), as in cardiovascular evaluations, are used to detect gastrointestinal bleeding and to determine the RBC mass in patients. RBC have been labeled with 99mTc through in vitro, in vivo or in vivo/in vitro techniques (2, 9). 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. It is suggested that oxidative stress may result from the exposition to some drugs, ionizing radiation and deficiency of folic acid (10). 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 (11).

It has been stated that cardiovascular disease (CVD) and diabetes are growing public health burdens, and remain one of the leading causes of morbidity and mortality in Canada (12). It has become increasingly evident that individuals who present a cluster of metabolic disorders, known as the metabolic syndrome, are disposed to develop both CVD and type 2 diabetes. Some studies suggested that maternal diabetes can affect the embryology environmental, which could help to elucidate that the oxidative stress may be related to the disturbance of the gene expression which is essential in the control of the ontogenetic processes. Aging is accompanied by decreased specific activity in many enzymes, altered heat stability, and increased carbonyl content of proteins (13). The nonenzymatic 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 implied in diabetes, eye disorders, and amyloid accumulation. Many extracellular matrix proteins exhibit increased cross-linking with age. The characterization of molecular 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 (14).

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 (15). In this study, we have evaluated the influence of a chayote extract and diabetic on the labeling of blood constituents with 99mTc using an in vitro technique.

Material and Methods

Preparing and analysis of the extract
Chayote was purchased from a local market in Rio de Janeiro city, RJ, Brazil. To prepare the extract, 50 g of skin of chayote 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. They were not found in the extracts of chayote used in the experiments. The method used to verify the presence of these toxic products is based on inhibition of acetylcholinesterase in the presence of the pesticides (16). In this method, brain acetylcholinesterase is utilized as an in vitro detector of organophosphorus and carbamate insecticides. Briefly, a preparation of acetylcholinesterase was obtained from the extraction of a rat brain microsomal fraction with Triton X-100 and was incubated with the extract of chayote. 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 chayote)

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 high quantities of sugar in their drinking during 1.5h. After 48h of the induction it was performed the rate of sugar tests by tail punition. It was considered diabetic the rats with rate of sugar rates above 180 dg/dL.

Bioavailability Experimental
The experiments were performed with the chayote extract administrated to the animals. The plant extract was prepared in the concentration of 0.1 g.mL⁻¹ and it was used the skin of the chayote. The animals have been divided into 4 groups (control, diabetic, normal treated with chayote extract and diabetic treated with the chayote extract), each group with 6 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. 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 ratio of radioactivity (%ATI) was calculated. Statistical analysis (Dunnett test) was performed to compare the experimental data.

Diabetes Induction
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Results and Discussion
Therapeutic drug may be capable to modify the nature/amount of the 99mTc-radiopharmaceutical bound to the blood elements, which may result in unexpected behavior of the radiopharmaceutical. Therapeutic drugs and extracts of medicinal plants can also alter the labeling of blood elements with technetium-99m (17, 18). We agree with (1) in that many 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 both drug/radiopharmaceuticals interactions and the consequences for the bioavailability of the radiopharmaceuticals and the labeling of blood constituents (9).

In an in vivo study, it is described that chayote extracts (macerated and decoct) were capable of altering the labeling of blood elements with 99mTc. (19). An extract from cauliflower leaves administrated to the animals during the same time was not capable to alter the radiolabeling of blood elements, as it has been reported (20). The labeling process of blood elements with 99mTc needs a reducing agent, and probably the stannous ion would be oxidized. In in vitro studies it was verified that extracts of Thuya occidentallis (5), Nicotiana tabacum (21) and Maytenus ilicifolia (22), could have oxidative compounds, and the labeling of blood elements decreases in the presence of these extracts. It has been reported that Sechium edule extract is capable of altering the biodistribution of 99m-Tc-radiopharmaceutical (23).

Many properties are attributed to the chayote; one of them is the hypotensive effect (7) due to the action of metabolites produced by the metabolism of chayote. The diuretic effect (3) may support the action of chayote described in (7). In this study it has been observed an increase in the radiolabeling of blood cells and in the insoluble fraction of the blood cells isolated from the animals treated with the referred extract, and in diabetic rats in comparison to the diabetics animals isolated from the animals treated with the referred extract. (24) has demonstrated that chayote extracts are capable of altering the bioavailability of sodium pertechnetate, although (25), in other research with in vitro experimental, has related that chayote extract had an antioxidant effect.
The genotoxic effect of *Paullinia cupana* (26) and *Brassica oleracea* (cauliflower) (20), both natural products, could be associated to the generation of reactive oxygen species (ROS) that are oxidative agents. It has been 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 radical attack. Reactive oxygen species appear to play a role in regulating differential gene expression. It is known that in diabetic it is observed a decrease in the
pH of blood (27). In this study we can hypothesize that this fact could be associated with the increase on the labeling of blood cell and cell proteins with 99mTc, considering that blood cells may participate in the control of blood pH. Alterations on the shape of the red blood cells were found on blood treated with tobacco (21), *Sechium edule* (23) and *Maytenus ilicifolia* (5). These studies may support an action of the extract in the cell membrane. It has been verified that 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 (14). This fact may support an increase of the %ATI linked to blood proteins taking into account 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, we should be careful when surpassing experimental data to the clinical situation, since the observed effects may depend on the amounts of the drug used (28).

**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 alters the radiolabeling of blood constituents. In this case, it is suggested 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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**References**


