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Evaluation of the Effect of an Echinacea Extract in Rats: Study of the Mass of Organs And of the Bioavailability of the Radiopharmaceutical Sodium Pertechnetate (Tc-99m)

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Abstract: Natural or synthetic drugs can alter the bioavailability of radiopharmaceuticals used in diagnostic imaging in nuclear medicine. The knowledge of such altered bioavailability is important in making diagnostic of diseases and to try to understand biological effect of drugs in specific organs. The use of natural products has been increasing in the world. Echinacea is a natural product widely used for the treatment of respiratory tract infections. The aim of this study was to evaluate the effect of the echinacea on the bioavailability of the radiopharmaceutical 99mTc-sodium pertechnetate ($Na^{99m}TcO_4$) and on the mass of organs to try to verify possible undesirable actions of the studied natural product. An extract of Echinacea was daily administered by intragastric via into *Wistar* rats during 10 days. $Na^{99m}TcO_4$ (3.7MBq) was administered by ocular plexus via and 10 minutes after the animals were sacrificed. Blood and various organs were removed, their mass determined, their radioactivity counted in a well counter and the percentages of the injected dose per gram of organ (%ID/g) calculated. The results showed that in the treated animals: (i) the mass of the organs was not modified; (ii) the %ID/g decreased in lungs. The effect of echinacea on the $Na^{99m}TcO_4$ bioavailability in the lung was statistically significant (Student test, $p<0.05$) and it could be explained by the metabolization or therapeutic action of this herb in this organ.

Key words: Echinacea, $Na^{99m}TcO_4$, bioavailability, mass

Introduction

The use of radionuclides for medicine and for basic and clinical researches has grown at a very rapid place. Procedures based on their use as radiopharmaceuticals for nuclear medicine imaging and for radiotherapy of cancer and other diseases, have become firmly established as important clinical modalities (Srivastava, 1996).

In nuclear medicine radiopharmaceuticals are internally administered and the diagnostic of the disease is gained by recording the distribution of the radioactive material in both time and space. The pharmacokinetics of the radiopharmaceuticals and the selective tissue uptake are the bases of diagnostic utility (Thrall and Ziessman, 1995).

The radionuclide technetium-99m (99mTc) has excellent physical characteristics for scintigraphic imaging in nuclear medicine and also is inexpensive. A variety of chemicals and cellular structures has been labeled with this radionuclide to be utilized as radiopharmaceuticals (Chandra, 1998). 99mTc is easily obtained by sterile 99Mo/99mTc generator, an apparatus containing 99Molibidenium (99Mo) radioisotope adsorbed on alumina which provides sterile, pyrogen-free 99mTc as sodium pertechnetate eluate ($Na^{99m}TcO_4$). The eluate can be administered directly or serves for labeling purposes (Owunwanne *et al.*, 1995).

$Na^{99m}TcO_4$ is one of the common radiopharmaceutical. Its biological behavior is similar to iodine and localizes in the thyroid, salivary glands, gastric mucosa and choroid plexus of the brain. Following intravenous injection, part of the $Na^{99m}TcO_4$ becomes bound to plasma proteins. The plasma disappearance of $Na^{99m}TcO_4$ is very rapid and it is secreted by the gastric mucosa in the stomach and the intestine. Aproximately 30% of the injected activity is excreted in the urine in the first 24 hours. Imaging is performed 30 to 180 minutes after injection with a collimated NaI(Tl) detector (Saha, 1998.).

In the presence of biochemical and/or pathophysiological changes, the normal bioavailability of a radiopharmaceutical may be altered. This altered biologic behavior due to diseases helps a physician to define a diagnosis of a possible disease. Altered biological behavior may also be due to interference caused by pharmacodynamic effects of natural or synthetic drugs. If unknown, the drug interaction with radiopharmaceuticals

can lead to misdiagnosis or the necessity to repeat the examination, increasing the radiation dose to the patient. (Sampson, 1993). Moreover, the evaluation of the bioavailability of the radiopharmaceutical is a worthwhile tool to study undesirable action of different products and to suggest possible located biological effect , as previously reported (Diré *et al.*, 2001; Moreno *et al.*, 2002 and Mattos *et al.*, 1999).

Medicinal plants are widely used worldwide for the treatment of many diseases. Sometimes the toxic effects of these products are not fully known. Many natural products have been report to affect the biodistribution of different radiopharmaceuticals used in diagnostic imaging in nuclear medicine. Furthermore, the alterations on the bioavailability of radiopharmaceuticals could be due to the toxic effect of substances in an organ (Diré *et al.*, 2001; Moreno *et al.*, 2002 and Mattos *et al.*, 1999).

Echinacea (*Echinacea purpurea*) is the name of a genus of native North American plants, commonly known as the purple coneflower. Echinacea plant extract is widely used for the prevention and the treatment of upper respiratory tract infections (Melchard *et al.*, 1998). Most uses of echinacea are based on the reported immunological properties (Barrett, 2003).

The purpose of this study was to evaluate the effect of the medicinal herb echinacea on the bioavailability of the $Na99mTcO_4$ and on the mass of isolated organs from treated Wistar rats.

Materials and Methods

We have used an animal model with female *Wistar* rats (body mass 300g) to evaluate the herb interference. The animals were divided into 2 groups: experimental ($n=4$) and control group ($n=4$). In the experimental group, a commercial sample Of Echinacea (Herbarium Laboratório Botânico, Brazil, lot 266512, validity Jan/2005) solution (36mg/mL) was daily administered by intragastric via during 10 days, in according with the European Guide of Animal Practice (Diehl *et al.*, 2001). In the control group, saline solution (0.9% NaCl) was administered by the same via. One hour after the last dose, 0.3mL of the tracer solution ($Na99mTcO_4$ 3.7 MBq) were injected in each of ten rats via ocular plexus. The $Na99mTcO_4$ was recently eluted from 99Mo/99mTc generator (Instituto de Pesquisas Energéticas e Nucleares, Brazil). The rats were rapidly sacrificed at 10 min post injection of the radiopharmaceutical. Samples of blood were collected and the various organs (pancreas, thyroid, brain, ovary, spleen, kidney, heart, stomach, lung, liver, thin intestine, muscle and bone) were isolated. The mass of the organs were determined in an analytical balance (Analytical Plus Electronic Balances-Ohaus Corporation, model AP250D-USA) and the 99mTc radioactivity in each organ was determined in a gamma counter NaI(Tl) (Automatic Gamma Counter, 1272 Clinigamma, LKB, Wallac, Finland). The percentage of the injected dose per gram (%ID/g) of $Na99mTcO_4$ was calculated dividing the injected dose in each organ by the mass of the organ and multiplied for 100. The samples of blood was immediately centrifuged (700 x g, 10 minutes) to obtain plasma. The plasma obtained was used for the spectrophotometric estimation of creatinine, urea (Microlab 100 Chemical Kit Emergencial, USA) and glucose concentrations (Bio System BTS310 Photometer, USA), using commercial kits (Urea UV Cinetica AA, Creatinine Cinetica AA and Glicemia enzimatica AA from Wiener Lab, Argentine). The results were compared with the control group and statistical analysis were performed by Tukey test ($p<0.05$) to determine the significance of the difference between experimental and control groups.

Results

Table 1 shows the relationship between the mass of organs of the experimental and control groups. The analysis of the results reveals no significant alterations ($p<0.05$) of the mass of the organs studied.

Table 2 shows the relationship between the %ID/g of $Na99mTcO_4$ in the experimental and control group. The analysis of the results reveals significant reductions ($p<0.05$) of the %ID/g in lungs and no significant alteration in pancreas, ovary, spleen, thyroid, thin intestine, heart, brain, liver, bone, muscle, stomach, kidney and heart.

As shown in table 3 the plasma concentrations of urea, creatinine and glucose in the experimental group were not significantly different ($p<0.05$) from the control group.

Discussion

The administration of a radiopharmaceutical results in its selective localization in the organ or organs of interest in a patient (Chandra, 1998). A marked deviation in the expected biodistribution of a radiopharmaceutical may occur as a result of the medication interfering with the disposition of the radiopharmaceutical (Sampson, 1993). When this modified biologic behavior is desired, the alteration is used for diagnostic intervention or drug therapy monitoring (Hesslewood and Leung, 1994). The undesired altered bioavailability of the radiopharmaceutical may provide misleading information that can either mask or mimic certain disease symptons (Gomes *et al.*, 2002 and Chen *et al.*, 1994). In this case, the drug should have an undesirable and unknown biological action in an organ (Moreno *et al.*, 2002; Mattos *et al.*, 1999 and Gomes *et al.*, 2002).

Many drugs alter hormonal status and these effects may produce marked deviations from expected biodistribution

Table 1: Effect of Echinacea on the mass (g) of different organs from female Wistar rats

	Groups	
Organs	Control group	Experimental group
Bone	0.523 ± 0.092	0.651 ± 0.087
Brain	1.574 ± 0.056	1.596 ± 0.099
Heart	0.656 ± 0.025	0.706 ± 0.079
Kidney	0.654 ± 0.015	0.671 ± 0.088
Liver	6.553 ± 0.538	6.641 ± 0.864
Lungs	0.313 ± 0.093	0.367 ± 0.079
Muscle	1.080 ± 0.262	1.198 ± 0.298
Ovary	0.135 ± 0.024	0.123 ± 0.336
Pancreas	0.349 ± 0.007	0.387 ± 0.063
Spleen	1.212 ± 0.250	1.123 ± 0.336
Stomach	1.013 ± 0.092	1.046 ± 0.142
Thin intestine	0.366 ± 0.062	0.372 ± 0.089
Thyroid	0.098 ± 0.019	0.089 ± 0.022

The organs were isolated and their mass determined in an analytical balance. The results of the experimental group (mean = 4) were compared with the control group (mean = 4), and statistical analysis were performed with Tukey test ($P < 0.05$). The values are averages ± standard deviations

Table 2: Effect of Echinacea on the percentage of injected dose per gram of $Na99mTcO_4$ in female *Wistar* rats

	Groups	
Organs	Control group	Experimental group
Blood	0.125 ± 0.042	0.115 ± 0.028
Bone	0.465 ± 0.083	0.548 ± 0.114
Brain	0.219 ± 0.044	0.194 ± 0.399
Heart	1.310 ± 0.187	1.131 ± 0.521
Kidney	0.099 ± 0.021	0.096 ± 0.028
Liver	0.102 ± 0.014	0.109 ± 0.022
Lungs	2.564 ± 0.464	1.412 ± 0.498
Muscle	0.342 ± 0.072	0.295 ± 0.036
Ovary	3.289 ± 0.187	3.249 ± 0.469
Pancreas	4.111 ± 0.612	3.721 ± 0.829
Spleen	0.293 ± 0.074	0.395 ± 0.089
Stomach	0.168 ± 0.033	0.227 ± 0.082
Thin intestine	17.424 ± 1.816	18.732 ± 4.363
Thyroid	3.882 ± 0.742	4.037 ± 0.499

The percentages of injected dose per gram (%ID/g) were determined for each organ. The results of the experimental group (mean = 4) were compared with the control group (mean = 4). Statistical analysis were performed with Tukey test ($p < 0.05$). The values are averages ± standard deviations.

(Sampson, 1993). It was described in the literature many drugs that are capable to alter the bioavailability of different radiopharmaceuticals labeled with 99mTc (Hesslewood and Leung, 1994). Using an animal model, (Mattos *et al.*, 1999) related an alteration in the uptake of the 99mTc-MDP caused by vincristine, a vinca alkaloid that is used as chemotherapeutic drug. This same drug was capable to alter the mass of the thymus, spleen and lymph nodes and also altered the %ID/g of the 99mTc-glucoheptonic acid (99mTc-GHA) in these cited organs (Mattos *et al.*, 2001). Mitomycin-C, other chemotherapeutic drug, alters the bioavailability of various 99mTc-radiopharmaceuticals in mice, including 99mTc-diethylenetriaminepentaacetic acid (99mTc-DTPA), 99mTc-dimercaptosuccinic acid (99mTc-DMSA) and 99mTc-GHA employed in renal evaluations (Gomes *et al.*, 2001), 99mTc-pyrophosphate (99mTc-PYP) (Gomes *et al.*, 2002) and 99mTc-MDP (Gomes *et al.*, 1998) used in bone imaging and 99mTc-phytic acid (Gomes *et al.*, 2002) used in hepatic scintigraphy, and $Na99mTcO_4$ (Gomes *et al.*, 2002). Glucantime, a drug with antileishmanial activity, has induced an altered distribution of the bone scanning agent 99mTc-MDP (Holanda *et al.*, 2002). Chayotte (*Sechium edule*) a subtropical vegetable with potent diuretic action and very useful as food, has altered the bioavailability of the $Na99mTcO_4$ (Diré *et al.*, 2001). Eggplant (*Solanum melongena*) a purple fruit used in popular medicine in the treatment of hepatitis has also modified the

Table 3: Biochemical analysis of plasma compounds from *Wistar* rats blood

Compounds (mg dL^{-1})	Control	Experimental
Urea	46 ± 7.2	45 ± 5.6
Creatinine	0.5 ± 0	0.5 ± 0
Glucose	137 ± 17.6	138 ± 25.8

The results of the experimental group (mean = 4) were compared with the control group (mean = 4). Statistical analysis were performed with Tukey test ($P < 0.05$). The values are averages ± standard deviations.

uptake of the $Na99mTcO_4$ in the liver (Capriles *et al.*, 2002). *Ginkgo Biloba* a medicinal plant utilized to improve the memory and the attention, has affected the %ID/g of the $Na99mTcO_4$ in kidneys, liver and thin intestine (Moreno *et al.*, 2002).

No significant herb-drug interactions with echinacea have been reported. Adverse effects reported generally have been uncommon and minor, including abdominal upset, nausea, and dizziness (Kligler, 2003). In this study was observed that echinacea was capable to alter the %ID/g of the $Na99mTcO_4$ in lungs. Some authors have reported that alkylamides are one of the active constituents of echinacea plant that significantly increase the phagocytic activity as well as phagocytic index of the alveolar macrophages. The immunomodulatory effects of alkylamides appear to be more pronounced in lungs than in spleen (Goel *et al.*, 2002). This fact would explain the alterations provoked by echinacea, in the uptake of the Na99mTcO4 in lungs.

Echinacea was not able to modify the mass of the organs studied and the effect of this herb on the bioavailability of the studied radiopharmaceutical in the lungs and it could be justified by the metabolic process or the therapeutic effect of echinacea and by the characteristics of the radiopharmaceutical. Moreover, it is possible to speculate that this herbal medicine is capable to induce physiological/structural modifications in lungs.

Due to the capacity of the natural or synthetic drugs to alter the biodistribution of 99mTc-radiopharmaceuticals, we are suggesting the use of this experimental model to evaluate the biological effect of drugs in the different organs. Moreover, although our results were obtained with animals, we suggest to care attention with the examination in nuclear medicine in patients under the medication treatment.

Conclusion

The effect of echinacea on the $Na99mTcO_4$ bioavailability could be explained by the therapeutic action of this herb. The development of biological models to study the drug/radiopharmaceutical interaction is worthwhile, as well as to understand better the biological action of natural and synthetic products.

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