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A N I N T E R N A T I O N A L J O U R N A L

Effect of Paclitaxel (Taxol®) on the Biodistribution of Sodium Pertechnetate ($Na^{99m}TcO_4$) in Female *Wistar* Rats

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ABSTRACT

The evidence that natural or synthetic drugs can affect the biodistribution of radiopharmaceuticals (radiobiocomplexes) in setting of nuclear medicine clinic is already known. We studied the effect of Paclitaxel, an anti-neoplastic agent for the treatment of solid tumors, on the biodistribution of $Na^{99m}TcO_4$ in female rats. Paclitaxel (1mg/mL/week) was administered into animals in single dose during 3 weeks, with interval of 1 week among them. The control group received NaCl 0.9% solutions by the same via. One hour after the last dose, it was injected $Na^{99m}TcO_4$ in the animals. The percentage of activity per gram (%ATI/g) and biochemical and hematological determinations were performed. A significant increase were found in alanine aminotransferase, aspartate aminotransferase, glucose and in the %ATI/g of some organs (ovaries, uterus, vagina, breasts, large intestine and liver).These results can be associated, probably, to the capacity of paclitaxel to alter the biodistribution of $Na^{99m}TcO_4$ and the metabolism of glucose and hepatic enzymes.

Keywords: paclitaxel, drug interaction, radiopharmaceuticals, $Na^{99m}TcO_4$, antitumoral drug, cancer.

INTRODUCTION

Cancer is a multifactorial disease that results fromthe interaction of multiple genetic and environmental factors. Chemotherapy is usually given early after diagnosis in several cancer subtypes to offer best results (Steinkellner et al., 2001; Vaclavikova et al., 2003). The use of phytotherapeutic products by the world population has greatly increased in the last decades (Briskin, 2000; Ang-Lee et al., 2001; Chan, 2003). Paclitaxel, commercially known by Taxol®, is a compound with intense antitumoral activity extracted from the *Taxus* species. It is presently one of the most important drugs used in cancer

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chemotherapy (Itoh et al. 2004; Feldweg et al., 2005).

Paclitaxel is an unique anticancer agent with tubulin-stabilizing action, and widely used for several malignancies, including ovarian, breast, stomach and non-small cell lung cancers (Sparreboom et al., 1997; Akerley, 2000; Souza, 2004; Itoh et al. 2004; Feldweg et al., 2005).

However, chemotherapeutic drugs are often associated with some degree of toxicities, which are caused by reactive metabolites generated by the biotransformation of anticancer drugs in the liver (Steinkellner et al., 2001; Lahowel and Fillastre, 2004; Choi and Li, 2005). Paclitaxel is mainly metabolized through the liver and undergoes biliary excretion (Itoh et al. 2004; Feldweg et al., 2005).

Traditional noninvasive imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) are used primarily for imaging anatomical and morphological changes associated with diseases (Vallabhajosula, 2007). Historically, CT has been the modality of choice for the diagnosis and staging of malignant disease and for monitoring the response to treatments (Vallabhajosula, 2007).

These screening techniques, however, often lack the necessary sensitivity and specificity for early diagnoses of many cancers and for the detection of subcentimeter neoplasms and preneoplastic disease (Hanahan and Weinberg, 2000; Vallabhajosula, 2007). To develop effective treatment modalities, especially, patient specific treatments, a more sensitive and specific detection of early malignancies is essential. Molecular imaging is the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in a living system (Schlyer, 2003; Vallabhajosula, 2007). Radioisotope based molecular imaging techniques, such as positron emission tomography (PET) (Nutt, 2002; Rajendran and Mankoff, 2007) and single photon emission computed tomography (SPECT), capture functional or phenotypic changes associated with disease (Mankoff et al., 2005). It is a field that aims to integrate patient-specific and disease-specific molecular information with traditional anatomical or structural imaging readouts. The hybrid or fusion-imaging of PET/CT is improving the sensitivity and specificity of clinical PET imaging technique (Schlyer, 2003; Rajendran and, Mankoff, 2007).

The progress in diagnostic nuclear medicine over the years since the discovery of technetium-99m (^{99m}Tc) is indeed phenomenal. The preeminence of ^{99m}Tc is attributable to its optimal nuclear properties of a short half-life (6 hours), metastable radionuclide, radiotracer with gamma photon emission of 140 keV, which is suitable for high-efficiency detection for imaging with gamma cameras used in nuclear medicine and which results in low radiation exposure to the patient (Saha, 2004; Bernardo-Filho et al. 2005). The evidence that natural and/or synthetic drugs can affect the biodistribution of radiopharmaceuticals (radiobiocomplexes) in setting of nuclear medicine clinic is already known (Xavier-Holanda et al., 2002; Saha, 2004; Bernardo-Filho et al. 2005; chemotherapeutic treatment. This fact can to lead a misdiagnosis or unnecessary exposure to radiation during the repetition of these exams. Frequently, this phenomenon is responsible for modification of the biodistribution of the radiopharmaceutical (Xavier-Holanda et al., 2002; Thrall and Ziessman, 2003; Bernardo-Filho et al. 2005; Holanda et al., 2006). The aim of this study was to evaluate the effect of the paclitaxel on the biodistribution of the radiopharmaceutical sodium pertechnetate ($Na^{99m}TcO_4$) labeled with technetium-99m, in female *Wistar* rats and on some biochemical and hematological determinations.

MATERIALS AND METHODS

The animals were obtained from *Centro de Ciências da Saúde, Universidade Federal do Rio Grande do Norte,* Natal-RN, Brasil, were housed in groups with free access to food and water, maintained under constant environmental conditions (23±2º, 12h/12h of light/dark cycle). Studies were performed in healthy female *Wistar* rats (weight range: 180–250 g). Twelve animals were used in this experiment and were randomly divided into two groups (treated and control) of 6 animals each one. These experiments were approved by the Ethical Committee for Using Animals of UFRN, with the number CEA/212/2008.

Paclitaxel was kindly provided by Bristol-Myers-Squibb (30 mg paclitaxel in 6 ml of ethanol: CremophorEL, 50:50) and stored at 4ºC during use. Stock solutions of paclitaxel were prepared by dilution in methanol and stored at room temperature for a week.

In the treated group, paclitaxel, dissolved in isotonic saline solution (NaCl 0.9%) was administered by intraperitoneal via (IP) into animals at a dose of 1mg/mL/week, in single dose during 3 weeks, with interval of one week among them. The control group received saline solution by the same way and period. One hour after the last dose, it was injected 0.1 mL of $Na^{99m}TcO_4$ (3.7 MBq) via orbital plexus. $Na^{99m}TcO_4$ was eluted in a $^{99}Mo/^{99m}Tc$ generator (*Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brasil*). After 60 minutes, all animals were quickly killed under anesthesia with xylazine (20 mg/kg) and ketamine (50 mg/ kg), by IP via. Breast, large intestine, liver, ovary, oviduct, stomach, spleen, Santos-Filho and Bernardo-Filho, 2005; Holanda et al., 2006). These interactions can alter results of thyroid, uterus, vagina and samples of blood were isolated. The tissue were washed in saline, weighed in a balance (Mark 160®, Bel equipment, Italy) and the radioactivity was determined in an automatic gamma counter (Wizard 1470, Perkin-Elmer, Finland) and the percentage of radioactivity per gram of tissue (%ATI/g) was calculated. Before the administration of $Na^{99m}TcO_4$, it was withdrawn 2 mL of whole blood of each animal and the biochemical and hematological determinations were performed in automated equipment TermoKonelab 60i/Abbott and Cell-Dyn 3500R/Abbott, respectively. Data were presented as mean ± standard deviation. The percentage of radioactivity per gram (%ATI/g) was determined by dividing the percentage of total radioactivity of each tissue by its weight in grams. The ATI%/g was compared using the non-parametric Mann-Whitney test and the biochemical and hematological parameters by Student's t-test, considering the level of statistical significance at $p<0.05$ in both tests. Statistica 6.0 software was used.

RESULTS

Table 1 shows the relationship between the uptake (%ATI/g) of the $Na^{99m}TcO_4$ on the paclitaxel-treated group (n=6) and on the saline-control group (n=6), 60 min after administered of the $Na^{99m}TcO_4$. The analysis of the results shows a significant ($p<0.05$) increase of the uptake of radioactivity in breasts, large intestine, liver, ovaries, uterus and vagina.

Table 1 - Effect of paclitaxel treatment on the biodistribution of sodium pertechnetate activity in female *Wistar* rats after 60 min injection of radiopharmaceutical ($Na^{99m}TcO_4$).

Organs	% ATI/g	
	Controls	Treated
Blood	0.030±0.001	0.050±0.001
Breast	0.040±0.000	0.420±0.005*
Liver	0.160±0.002	0.360±0.003*
Large Intestine	0.050±0.000	0.150±0.004*
Ovaries	0.040±0.000	0.160±0.002*
Oviducts	0.080±0.001	0.090±0.002
Spleen	0.080±0.000	0.100±0.004
Stomach	1.110±0.006	1.280±0.008
Thyroid	3.190±0.010	3.280±0.013
Uterus	0.070±0.000	0.210±0.004*
Vagina	0.030±0.000	0.200±0.001*

Mean±SD. *, $p<0.05$

Table 2 shows the effect of the paclitaxel on the biochemical and hematological parameters of the female *Wistar* rats (n=6) and on the control group (n=6), before the administration of the $Na^{99m}TcO_4$. The analysis of the results shows a significant ($p<0.01$) increase of the glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and white blood cells.

Table 2 – Effect of paclitaxel treatment on biochemical and hematological parameters of female *Wistar* rats.

Biochemical and hematological parameters	Groups	
	Controls	Treated
ALT (UI/L)	110.17± 25.18	158.33± 16.91**
AST (UI/L)	111.17± 35.72	157.83± 52.15**
Glucose (mg/dL)	97.83± 14.96	154.67± 33.61**
Hematocrit (%)	29.76± 4.08	31.45± 3.42
Hemoglobin (g/%)	11.62± 1.01	11.88± 1.02
Leukocytes (u/mm^3)	1860.00± 338.20	3040.00± 701.90**
Lymphocytes (%)	53.33± 9.37	69.66± 4.92
Neutrophils (%)	54.83± 7.05	64.83± 5.56
Platelets (u/mm^3)	648167± 106878	669500± 111159
Red blood cells (u/mm^3)	5688333± 889436	5746667± 646209
Total proteins (g/dL)	6.66± 0.78	6.75± 0.52

Mean±SD. **, $p<0.01$.

DISCUSSION

Cancer is a systemic disease resulting from alterations in the interactions between oncogenes and tumor suppressor genes, which under normal physiological conditions control cell maturation, division and migration (Hanahan and Weinberg, 2000; Steinkellner et al., 2001; Vaclavikova et al., 2003).

In our study, we observed alterations on biochemical and hematological parameters and on biodistribution of the radiopharmaceutical sodium pertechnetate in female *Wistar* rats treated with the paclitaxel in some tissues.

Paclitaxel is an antineoplastic agent that has shown great promise in the therapeutic treatment of certain solid tumors including breast cancer. It is also the most promising anti-mitotic agent developed for cancer treatment in the past decade (Rosenblum and Shivers, 2000). The primary mechanism of action of paclitaxel is attributed to its ability to bind to microtubules and prevent their assembly. All treatment regimens for majority of cancers produce a lot of side effects, including hematological or liver toxicities (Steinkellner et al., 2001; Lahowel and Fillastre, 2004).

ALT and AST are diagnostic tumor markers in liver and heart diseases. The decreased activities of these enzymes in liver indirectly indicate the progression of tumor growth as tumor markers are directly associated with the malignancy in the cancerous conditions and is a potential molecular biomarker for assessing exposure to any toxic agents (Boutet et al., 2005). Tissue damage is the sensitive feature in the cancerous conditions so any deterioration or destruction of the membrane can lead to the leakage of these enzymes from the tissues. Hence, the elevation of these liver specific enzymes observed in breast cancer condition may be due to the progression of tumor growth (El-Beshbishy, 2005). Itoh et al. (2004) have reported increased activities of the enzymes (ALT and AST) in plasma and serum of cancer bearing rats, and now, our experiments also demonstrated increased activities of these enzymes (ALT and AST) in the chronic treatment of female rats with paclitaxel.

We have previously shown that the antiparasitic drugs such as glucantime and mefloquine (Xavier Holanda et al., 2002; Holanda et al., 2006) can alter the biodistribution *in vivo* of ^{99m}Tc-methylenediphosphonic acid (^{99m}Tc-MDP) in *Wistar* rats. Besides these studies, it was also observed alterations on the biodistribution of the $Na^{99m}TcO_4$ in organs of *Wistar* rats treated with *Punica granatum* and *Artemisia vulgaris* (Amorim et al., 2003; Holanda et al., 2006). In our experiment, we demonstrated that the paclitaxel increased the uptake of $Na^{99m}TcO_4$ in ovaries, uterus, vagina and breasts of female *Wistar* rats treated with this drug, what suggest an action of this antineoplastic agent in these organs. The %ATI/g also increased in liver and large intestine. This fact probably occurred due to the paclitaxel to

be metabolized through the liver and undergoes biliary excretion (Itoh et al., 2004).

In conclusion, these experimental models permit to study drug interactions and biological activities of vegetal extracts and synthetic drugs. Moreover, these findings could be worthwhile to try to understand and to avoid some pitfalls in the nuclear medicine imaging.

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RESUMO

Já está bem estabelecido na literatura científica que produtos naturais ou sintéticos podem alterar a biodistribuição de radiofármacos. O objetivo desse estudo foi avaliar a influência do paclitaxel, um agente antineoplásico para tratamento de tumores sólidos na biodistribuição do pertecnetato de sódio em ratas *Wistar* e na determinação de componentes bioquímicos e hematológicos. Paclitaxel, comercialmente conhecido por Taxol® (1mg/mL/semana), foi administrado, intraperitoneamente, nos animais do grupo tratado, em dose única, por 3 semanas, mas com intervalo de uma semana entre elas. O grupo controle recebeu solução de NaCl 0,9%. Uma hora após a última dose de paclitaxel, os animais receberam 0,1 mL de $Na^{99m}TcO_4$ (3,7MBq) via plexo orbital. O percentual de radioatividade por grama (%ATI/g) e parâmetros laboratoriais foram determinados. Ocorreu um aumento significativo (p<0,05) do %ATI/g nos ovários, útero, vagina, mamas, intestino grosso e fígado. Os níveis de glicose sangüínea e das enzimas hepáticas (ALT e AST) também aumentaram significantemente (p<0,01). Esses resultados podem estar associados, provavelmente, à capacidade do paclitaxel em alterar a biodistribuição do $Na^{99m}TcO_4$ e o metabolismo da glicose e de enzimas hepáticas.

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