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Influence of the Morphine on the Kinetic of Biodistribution of Technetium-99^{m} Labeled with MDP in *Wistar* Rats

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Abstract: The biodistribution of radiopharmaceutical used in diagnostic imaging can be altered by a wide variety of factors. There are some evidences that some drugs (natural or synthetic) can modify the bioavailability of. radiopharmaceutical. If unknown, the drug interaction with radiopharmaceuticals can lead to misdiagnosis or the necessity to repeat the examination, increasing the dose to the patient. The morphine is the main analgesic drug of the group of the opium and it has been used extensively in the medical field to treat severe pain, mainly those provoked by cancer. Many patients suffering of oncologic pain utilize the morphine as anthialgic therapy and need to submit themselves to complementary exams like bone scintigraphy which utilizes the methylenediphosphonic acid(MDP) labeled with ^{99m} Technetium (^{99m}Tc-MDP) to evaluate the presence of metastasis. To evaluate the effects of the morphine on the biodistribution of ^{99m}Tc-MDP in male *Wistar* rats, morphine (30mg/Kg/day) was administered in male *Wistar* rats (n = 6; age = 3 months old),orally, during 7 days. One hour after the last dose, were injected 0,1ml of 99Tc-MDP(100iCi), via ophthalmic plexus. The control group (n = 6) received only physiologic solution, instead of morphine, in the same conditions. The animals were rapidly sacrificed in 2 different periods: one group in 60 minutes (n = 6) and the other group in a 120 minutes (n = 6) after administration the ^{99m}Tc-MDP. The various organs from the animals were isolated then, the radioactivity of the ^{99m} Tc-MDP was counted and compared between the two groups : morphine treated and control. Statistical analysis were performed using the Wilcoxon test($P < 0.05$) The analysis of the results reveals a significant increase ($p < 0.05$) of the uptake of radioactivity in the treated group at 60 minutes in lungs, heart , stout bowel, liver, spleen, kidneys, bladder, femoral muscles and femoral boneand a reduction in thyroid, stomach and pancreas. And in the group treated at 120minutes analysis of the results reveals a significant reduction ($p < 0.05$) of the %ATI in lungs, heart, thyroid, stout bowel, liver, pancreas, spleen, kidneys, bladder, testis, femoral muscles and bone, on the other hand , results show a increase of the %ATI in stomach and duodenum. These results could be associated with the biological effects or/and metabolization of the morphine.

Key words: Morphine, radiopharmaceuticals, drug interaction, biodistribution ^{99}TC-MDP, pain

Introduction

Many experimental models studies have been described to evaluate drugs in terms of its therapeutic actions, metabolism and drug interaction (Amorim *et al.*, 2003; Bernardo-Filho *et al.*, 1992; Bernardo-Filho, 1988; Bernardo-Filho, 1995; Bernardo-Filho *et al.*, 1994; Gomes *et al.*, 2002; Gutfilen *et al.*, 1992; Gutfilen *et al.*, 1996; Mattos *et al.*, 2000; Santos *et al.*, 1990 and Vidal *et al.*, 1998).The technology of biodistribuition of radiopharmaceuticals has an important hole in this context. In Brazil, studies performed by the Universidade Estadual do Rio de Janeiro(UERJ) demonstrate the influence of a variety of natural and synthetic drugs on the biodistribution of radiopharmaceuticals (Amorim *et al.*, 2003; Bernardo-Filho *et al.*, 1992; Bernardo-Filho, 1988; Bernardo-Filho, 1995; Bernardo-Filho *et al.*, 1994; Gomes *et al.*, 2002; Gutfilen *et al.*, 1992 and Gutfilen *et al.*, 1996). In this model, the 99^{m}-technetium has been the chosen radionuclide utilized due to its optimal physical properties: a) a short half-life (6 hours); b) low levels of gamma radiation(140keV) offering minimal dose to the patient; c) convenient availability from a 99Mo/99^{m}Tc generator ;d) good affinity to bind to blood elements and e) minimal

environmental impact (Early and Sodee, 1995; Gutfilen *et al.*, 1994 and Saha, 1998).
There is a need to review the kinetic of biodistribution of this radioactive element (technetium) in mammals. After an intravenous injection, the 99^m-technetium is up taken by salivary and thyroid glands, choroids plexus. It is also secreted by gastric mucosa (Dewanjee *et al.*, 1990; Early and Sodee, 1995; Hung *et al.*,1996 and Saha, 1998). When associated with drugs (e.g. radiopharmaceuticals) this biodistribution modifiesand the 99^m-technetium is taken to desired "target" organ. This is the principle of scintigraphic exams (Early and Sodee, 1995 and Saha, 1998). In specific case of methylenediphosphonic acid (MDP), the labeling with 99^m-technetium and its clinical uses are well-known since 1970 (Early and Sodee, 1995 and Saha, 1998). The presence of phosphoric group facilitates ionic exchanges with hydroxyapatite crystals of bone (Hladik *et al.*, 1982).
Patients suffering from oncologic bone pain need to submit themselves to diagnostic investigations utilizing bone scintigraphy, above all when the indication is primary bone or metastatic tumor (Early and Sodee, 1995; Foley *et al.*, 1995; Saha, 1998 and WHO, 2003).
About 18 million people around the world suffer from pain caused by cancer and 70% of the patients with cancer in an advanced stage complain pain as the main symptom (WHO, 2003).
High potency opiods are the most utilized drugs in the treatment of oncologic pain, being the morphine, the main representative of this group, utilized extensively in the combat of severe pain (Camargo, 2003; Fiscella *et al.*, 1986; Foley *et al.*, 1995; Goodman and Gilman, 1988; Spielgel *et al.*, 1990 and WHO, 2003).
The drug interaction with radiopharmaceuticals may lead to misvisualization in scintigraphic procedures, causing misdiagnosis or necessity to repeat the exam, exposing the patient to excessive and unnecessary radiation (Early and Sodee, 1995; Hesselewood and Leung, 1994; Hung *et al.*,1996; Saha, 1998 and Santos *et al.*, 1990).
So, due to the widely use of the morphine in the treatment of pain, mainly on the patient with cancer, who can be submitted more frequently to nuclear medicine procedures (diagnostic or therapeutic) as scintigraphy , we developed a study to evaluate the effects of the morphine on the biodistribution of ^{99m}Tc-MDP in male *Wistar* rats.

Materials and Methods

In this experiment was utilized a total of 12(n = 12) male, *Wistar* rats, with 3 months of age , being divided into two groups : treated (n = 06 animals) and control(n = 06 animals). The animals were healthy and had free access to water and standard food. The animals were in captivity under controlled temperature and luminosity in the bioterium at the Universidade Federal do Rio Grande do Norte.
These experiences were performed in compliance with guidelines (local and international regulations) on the use of living animals in scientific investigations. In the treated group (n = 06), morphine sulfate (Dimorf®10mg/ml) was administered by 30mg/kg/day , in a single dose, per mouth, through orogastric probe, after inhalatory sedation using ether at 50% into male *Wistar* rats, during 7 days. One hour after the last dose,0,1ml of 99m-Tc-MDP (7.4MBq) was injected by ocular plexus way. In the control group(n = 06) morphine was not administered and saline solution (NaCl 0,9%) was administered by the same way. To prepare the ^{99m}Tc-MDP, ^{99m}Tc, as sodium pertechnetate, recently milked from ^{99}Mo/^{99m}Tc generator (Instituto de Pesquisas Energéticas e Nucleares, Brazil) was added to a kit of MDP(Laboratório de Radiofarmácia, INCa, Brazil).
The animals were rapidly sacrificed in 2 different periods: one group in 60 minutes (3 treatedand 3 control , n = 06) and the other group in a 120 minutes (3 treated ,.3 control n = 6) after administered the ^{99m}Tc-MDP. The various organs from the animals were isolated (brain, heart, thryoid, lungs, kidneys, testis, stomach, intestines, pancreas, spleen, liver, femoral muscle, femoral bone and bladder) and put in vials and then, the radioactivity of the ^{99m} Tc-MDP was counted in a well counter NaI (Tl)(Automatic Gamma Counter,1272,Clinigamma,LBK,Wallac,Finland). The percentages of radioactivity (%ATI) in the organs were calculated dividing the activity in each organ by the total activity administered; The percentage of radioactivity in each organ was compared between the two groups: morphine treated and control. Statistical analysis were performed using the Wilcoxon test ($P < 0.05$).

Results

Table 1 has shown the relationship between the uptake (%ATI) of the ^{99m} Tc- MDP in the group of the rats that was treated with morphine (n = 3) and in the control group (n = 3), 60 minutes after administered ^{99m}Tc-MDP. The analysis of the results reveals a significant ($p < 0.05$) increase of the uptake of radioactivity in lungs, heart , stout bowel, liver, spleen, testis, kidneys, bladder, femoral muscles and boneand a reduction in thyroid, stomach and pancreas. The results also reveal no significant alteration of the %ATI in the brain and duodenum.
Table 2 has shown the uptake (%ATI) of the ^{99m}Tc-MDP in the group of rats treated with morphine (n = 3) and in the control group (n = 3), 120 minutes after administered ^{99m}Tc-MDP. The analysis of the results reveals a significant ($p < 0.05$) reduction of the %ATI in lungs, heart, thyroid, stout bowel, liver, pancreas, spleen, kidneys, bladder, testis, femoral muscles and bone, on the other hand , results show a increase of the %ATI in stomach and duodenum. The results also reveals no significant alteration of the %ATI in the bone tissue.

Table 1: Effect of the morphine on the kinectic of biodistribution of the 99^{m}Tc-MDP in *Wistar* rats sacrified after 60 minutes after the injection of the radiopharmaceutical

%ATI Órgans	Control	Treated
Bladder	3.23 ± 0.14	60.01 ± 77.86
Brain	0.33 ± 0.01	0.80 ± 0.48
Duodenum	5.86 ± 0.13	6.40 ± 3.31
Femoral Bone	0.49 ± 0.01	23.05 ± 2.61
Femoral Muscle	0.34 ± 0.03	7.26 ± 0.58
Heart	0.18 ± 0,01	5.42 ± 1.45
Kidneys	1.15 ± 0.71	63.90 ± 29.97
Liver	3.20 ± 0.17	44.90 ± 2.53
Lungs	0.22 ± 0.05	9.63 ± 6.06
Pancreas	3.63 ± 0.05	1.74 ± 0.55
Spleen	1.73 ± 0.03	12.09 ± 5.78
Stomach	39.30 ± 0.56	18.13 ± 4.64
Stout Bowel	0.98 ± 0.01	4.12 ± 1.31
Testis	0.18 ± 0.10	4.11 ± 2.83
Thyroid	55.33 ± 0.88	6.51 ± 0.89

Table 2: Effect of the morphine on the kinectic of biodistribution of the 99^{m}Tc-MDP in Wistar rats sacrified after 120 minutes after the injection of the radiopharmaceutical

%ATI Órgans	Control	Treated
Bladder	26.96 ± 0.29	2.48 ± 0.24
Brain	1.96 ± 0.21	0.45 ± 0.08
Duodenum	4.83 ± 0.04	7.33 ± 0.27
Femoral Bone	36.65 ± 2.02	37.44 ± 1.79
Femoral Muscle	9.12 ± 0.17	1.41 ± 0.03
Heart	18.13 ± 0.78	1.62 ± 0.28
Kidneys	10.32 ± 1.99	19.68 ± 2.58
Liver	25.61 ± 2.72	18.12 ± 1.59
Lungs	18.03 ± 8.55	2.57 ± 1.22
Pancreas	2.57 ± 0.04	1.31 ± 0.07
Spleen	7.31 ± 0.51	1.07 ± 0.05
Stomach	37.26 ± 0.39	40.36 ± 1.04
Stout Bowel	6.64 ± 0.05	3.09 ± 0.19
Testis	4.36 ± 0.12	0.72 ± 0.09
Thyroid	5.25 ± 0.04	4.54 ± 0.43

Morphine was administered by mouth in male *Wistar* rats(treated group). The control group did not receive morphine. The 99^{m}Tc-MDP was also injected in both groups. After 120 minutes the animals were raidly sacrificied, the organs isolated and the percentage of radioactivity(%ATI) determined in each organ. The values are mean ± standard deviation. The results of the treated group were compared with control groupand statistical analyses were performed by Wilcoxon test($p < 0.05$).

Discussion

The developing of models that permit the evaluation of. the biologic properties of natural and synthetic products is worthwhile. The pharmacokinetics of radiopharmaceuticals may be altered by a variety of drugs, disease sates and surgical procedures (Early and Sodee, 1995).

Some drugs enhance the localization of radiopharmaceutical in the target organs and other depress the uptake. The distribution, uptake, retention and the elimination of radiopharmaceuticals depend on several factors, such as regional blood flow tissue metabolism and the binding to the blood elements. If the drug interaction is unknown, the biodistribution of the radiopharmaceutical in the target organs could be altered and in consequence an undesired radiation dose distribution can be observed. In other consideration due to the influence of the drug a poor quality image of. the target organ could be obtained and/or a misdiagnosis is done (Hesselewood and Leung, 1994 and Sampson, 1993).

Technetium-99m labeled biphosphonates such methylenediphosphonic acid (MDP) have been used for bone scanning and for localization of primary bone and metastatic tumors. The uptake of ^{99m}Tc-MDP in bone reflects bone metabolism and blood flow. The high affinity of MDP for the calcium leads to diffuse the uptake of tracer

throughout the liver, muscles and bone (Early and Sodee, 1995 and Saha, 1998).
Morphine is the most effective opioid analgesic and the most widely used treatment for severe pain. (Camargo, 2003; Foley *et al.*, 1995 and Goodman and Gilman, 1988). Generally, morphine has a potent action on Central Nervous System, being a mixed of depressing and stimulating actions. In addition to that, it has a spasmodic properties over smooth muscular fibers (Gutfilen *et al.*, 1996; Goodman and Gilman, 1988). In its anthialgic action mechanism, morphine attach itself to the *MÜ* receptors, inhibiting adenilciclasis, leading to a reduction of AMPc, with subsequent closing of sodium canals and opening the potassium canals, generating a lower neuronal excitabilityand therefore slower conduction of pain stimuli (Camargo, 2003; Fiscella *et al.*, 1986; Goodman and Gilman, 1988 and Spielgel *et al.*, 1990).
Possibly, morphine penetrate directly into the brain (Camargo, 2003 and Goodman and Gilman, 1988) and may interfere on the biodistribution of the 99^{m}-technetium, reducing the uptake of radiopharmaceutical in these organ, as found in the present study in which occur reduction of %ATI after 120 minutes in the brain.
Morphine is well-absorbed by mucosa and by continuity solution on the skin. Although its absorption in the digestive system is slower (Early and Sodee, 1995; Fiscella *et al.*, 1986; Goodman and Gilman, 1988; Saha, 1998 and Spielgel *et al.*, 1990).This data could explain the increase of the %ATI in the stomach and duodenum only after 120 minutes observed in these study.
After its absorption, morphine goes through the blood and then distribute itself to the many organs, specially, liver, lungs, kidneys and muscle (Camargo, 2003 and Goodman and Gilman, 1988). Data also observed in this work, in which results show a increase of the %ATI in lungs, liver, heart, kidneys, bladder and stout bowel.
Morphine is biotransformated in hepatocytes by a microssomal enzyme. The referred drug as well as its metabolites (like normorphine) suffers a conjugate reaction becoming an inactive compound. Morphine is excreted through the kidneys(80%)within 6 hours, being completely eliminated in a 24 hours period. A small quantity is eliminate through the sweat and feces (10%) (Goodman and Gilman, 1988 and Hladik *et al.*, 1982). In this way, the metabolization process and the metabolites produced probably could contribute to a reduction in the uptake of ^{99m}Tc-MDP in these organs (liver and kidneys, respectively) (Early and Sodee, 1995; Goodman and Gilman, 1988; Hladik *et al.*, 1982; Saha, 1998 and Santos *et al.*, 1990). In the literature some authors have related similar results with the study of. the effects different drugs on the biodistribution of radiopharmaceuticals. Amorim *et al.* (2003), described that the extract of *Punica granatum* has been capable of altering the bioavailabity of ^{99m}Tc as sodium pertechnetate. In other study Xavier *et al.* (2002), related that glucantime has altered the biosdistribution of ^{99m}Tc-MDP. Similar to the results obtained in this research Gomes *et al.* (2002), using a different radiopharceutical, have reported that the mitomycin-C has promoted an alteration on the bioavailability of sodium pyrosphosphate (^{99m}Tc-PYP).

Conclusion

Due to the results obtained we may suggest that morphine interact with 99^{m} TC-MDP causing modifications in the uptake of such radiopharmaceutical in studied organs, possibly, owing to the biological effects, metabolization and/or therapeutic action of the referred drug. Moreover, although our results were obtained with animals, we suggest paying attention with examination in nuclear medicine in patients under the treatment with this painkiller.

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