Evaluation of the biological effect of brahmi (*Bacopa monnieri* Linn) extract on the biodistribution of technetium-99m radiopharmaceuticals

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Abstract

Natural products have been widely used by human beings in different medical and domestic applications. However, sometimes the biological effects of these products are not fully known. We investigated the effect of the oral ingestion of *Bacopa monniera* Linn (Brahmi) extract (BM) on the biodistribution of the radiopharmaceuticals i.e., ^{99m}Tc labeled ethylene dicysteine diethyl ester (^{99m}Tc-ECD) and ^{99m}Tc labeled cystine dimethyl ester (^{99m}Tc-CDM) in female Sprague Dawley rats. The rats (n = 5) were treated with BM extract (1 ml) and 0.9% NaCl solution (control, n = 5) for 8 days. After this period, ^{99m}Tc-ECD and ^{99m}Tc-CDM were injected and the rats were sacrificed rapidly. The organs (brain, heart, liver, lung, spleen, muscle, kidney, intestines and urine) were isolated and radioactivity in each organ was counted. The results showed that BM extracts altered the percentage of radioactivity of ^{99m}Tc-ECD and ^{99m}Tc-CDM in brain, heart, liver, lung, spleen, muscle, kidney, intestines and urine with respect to control. This finding was probably an example of drug interaction with a radiopharmaceutical, and a fact that could lead to misdiagnosis of the examination in clinical practice with unexpected consequences for the patient. [Life Science Journal. 2008; 5(2): 45 – 49] (ISSN: 1097 – 8135).

Keywords: ^{99m}Tc-ECD; ^{99m}Tc-CDM; *Bacopa monniera* extract; biodistribution

1 Introduction

Radiobiocomplexes known as radiopharmaceuticals, have been employed in nuclear medicine for many years for the diagnostic and/or treatment of diseases or to study the blood flow; morphology of organs; bioavailability and metabolisms of drugs (Chandra, 1998; Braga, 2000; Gomes, 1998, 2002). The use of radionuclides for medical and basic research applications has continued to grow at a rapid pace. Procedures based on the use of radiotracers for imaging and for radiotherapy have become established in clinical modalities. Unexpected patterns of radiopharmaceutical distribution provoke a flurry of inquiries regarding the quality of the administered agent (Dire, 2003; Early, 1996). The alterations in biodistribution may be related to a therapeutic drug interaction (Britto, 1998; Hladik III, 1987; Mattos, 2001; Oliveira, 2002; Amorim, 2003). The short half-life radionuclide, Technetium-99m (^{99m}Tc) is the most frequently used radionuclide in diagnostic nuclear medicine procedures for a wide variety of diseases (Giles, 1987; Vanlic-Razumenic, 1999; Srivastava, 1996).

The use of medicinal plants has increased in the last decades all over the world. *Bacopa monnieri* Linn (Brahmi) is a perennial creeping herb found throughout India. It is used as a nerve tonic in traditional medicinal system in India. Its ethanolic extract contains a mixture of triterpenoid, steroidal saponins designated as Bacosides A and B (Jyoti, 2006; Roodenrys, 2002). Bacoside A comprises a mixture of three saponins, bacogenin A1, A2 and A3, with A3 being a major constituent (Russo, 2005). Several other types of saponines have been isolated and characterized in the last few years (Russo, 2005). *Bacopa monnieri* (BM) extract has earlier been reported to augment both the cognitive functions and mental retention capacity in different behavior studies (Singh, 1997). Ethanolic extract of BM

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has been found to increase the activity of antioxidative enzymes in different brain regions of rat. This exhibits its antioxidative potential (Bhattacharya, 2000). It has been reported that ethanolic extract was also found to inhibit the amnesic effects of scopolamine, electroshock and immobilization stress (Singh, 1997) and can significantly improve the speed of visual processing, learning rate and memory consolidation (Russo, 2005). This extract also reduces the different stress effect in rat brain by Hsp70 expression, superoxide dismutase activity and P450 enzyme activity (Russo, 2005).

It has been reported that many chemotherapeutic drugs (natural or synthetic drugs) can alter the biodistribution and kinetics of radiopharmaceuticals (Hesslewood, 1994; Mattos, 1999). The incorporation of a radionuclide into a drug formulation permits the determination of biodistribution and kinetics. Mattos *et al*, using an animal model (Mattos, 1999), described vincristine as capable of altering the uptake of the ^{99m}Tc-methylenediphosphonic acid (MDP) in many organs. Britto *et al*, also reported that chemotherapeutic drugs (plant extracts) also alters the biodistribution of ^{99m}Tc-diethlenetriaminepentaacetic acid (DTPA) (Brito, 1998).

^{99m}Tc labeled ethylene dicysteine diethyl ester (^{99m}Tc-L,L-ECD) is a neutral, lipophilic tracer agent that crosses the blood-brain barrier easily and can be prepared in radiochemically pure and stable form. It is based on an ester derivatized bis(aminoethanethiol) tetraligand and shows high brain retention in human and nonhuman primates (Cheesman, 1989; Kung, 1984). ^{99m}Tc-L,L-ECD is metabolized to its two mono-acid mono-ester derivatives and to a lesser extent to the di-acid ^{99m}Tc-ethylene dicysteine (^{99m}Tc-EC). ^{99m}Tc-L,L-ECD is the most frequently used radiopharmaceuticals for human brain uptake and assessment of cerebral perfusion studies in nuclear medicine (Leveille, 1989; Hubert, 1998). Here we have used the ^{99m}Tc-ethylene dicysteine diethyl ester (^{99m}Tc-ECD) radiopharmaceutical for biodistribution of female Sprague Dawley rats (Walovitch, 1989). Cystine dimethyl ester (CDM) is also a diester form of cystine having a disulphide bond which is more stable than cysteine (Steinherz, 1982). It works closely with glutathione to remove toxins from different organs (liver, brain etc). Here we have also used the ^{99m}Tc-dimethyl ester of cystine (^{99m}Tc-CDM) radiopharmaceutical for biodistribution of female Sprague Dawley rats (http://www.anyvitamins.com/cystine-info.htm).

The study of drug (natural plant extract or synthetic) interactions with radiopharmaceuticals is highly relevant and desirable. The purpose of this work was to evaluate the influence of BM extract (ethanolic) on the biodistribution of the radiopharmaceuticals ^{99m}Tc-ECD and ^{99m}Tc-CDM in an experimental model using female Sprague Dawley rats.

2 Materials and Methods

2.1 Extract preparation

The whole BM plant was dried in shade and then crushed. The crushed materials were extracted with ethanol (Jyoti, 2007). This extract was dried in vacuo. The BM extract so prepared contained 55% – 60% bacosides-A. From this dried product, saline dilutions (0.9% NaCl) containing 100 mg/ml were prepared. This preparation was administered (oral dose was 100 mg/ml/day) to female Sprague Dawley rats (n = 5) during 8 days. The body weight of the rats were 300 – 350 mg. The control group (n = 5) received only solution of 0.9% NaCl during 8 days. Experiments were conducted in accordance with the Departmental Committee of Animal Ethics and with the Institutional Guidelines of Indian Institute of Chemical Biology, Kolkata, India.

2.2 Radiopharmaceutical preparation

^{99m}Tc, as $^{99m}TcO_4 \cdot Na$ used in the labeling procedure was obtained by 2-butanone extraction from a 5 N NaOH solution of 99Mo (Misra, 1994). Molybdenum-99/technetium-99m (kits from Board of Radiation and Isotope Technology (BRIT), Mumbai) generator was used for radiopharmaceutical preparation. ^{99m}Tc-ECD radiocomplex is prepared by standard kit available from Beijing Atom High Tech. Co. Ltd. This kit contains ECD (1.0 mg) and $SnCl_2$. Under sterile condition $^{99m}TcO_4 \cdot$ (1 – 2 ml) in 0.9% sodium chloride solution containing 6 – 8 mCi is added and shaken to dissolve the contents. ^{99m}Tc-ECD radiocomplex is prepared after 30 minutes of incubation at room temperature. CDM was procured from Sigma Chemicals Company, USA. ^{99m}Tc-CDM were prepared by reported standard method (Misra, 1994) using stannous chloride dehydrate ($SnCl_2$).

500 μCi of ^{99m}Tc-ECD/^{99m}Tc-CDM were administered through the femoral vein of anaesthetized rats with 0.5 mm polyethylene (PE) catheter in each rat of control and BM extract treated groups of rats. The animals were sacrificed after 2 minutes. It was established that at 2 minutes post injection (PI) the brain uptake of ^{99m}Tc-ECD is highest (Hubert, 1998). The radiochemical quality control was performed by chromatography. Labeling efficiency was > 95% and the percentage of free pertechnetate was < 5%. After 2 minutes the rats were quickly sacrificed and different organs (brain, heart, liver, lung,

spleen, muscle, kidney, intestines and urine) were isolated and weighted on a clinical scale and radioactivity was counted in a well-type counter (Gamma ray spectrometer model: GRS 23C, ECIL). Standard counts of the radiocomplexes were also taken and the uptake was expressed in percentage of radioactivity per gram of each organ (%ATI/g). Percentage of radioactivity in each organ was compared with the control group. Data were analyzed statistically by the *t*-test, with the level of significance set at $P < 0.05$.

3 Results

The effects of BM extract on the uptake of radiopharmaceuticals (^{99m}Tc-ECD/^{99m}Tc-CDM) in different isolated organs from BM extract treated and non-treated (control) groups of rats were shown in Tables 1 and 2. Table 1 showed the percentage uptake of ^{99m}Tc-ECD in the group of rats that was treated with BM extract and in the control group. The results revealed an increased and significant uptake ($P < 0.05$) of ^{99m}Tc-ECD in brain, liver, lung, intestines and urine after treatment with the BM extracts. Table 1 also showed significant decrease in the uptake of this radiopharmaceutical in the heart, kidney, muscle, and spleen after treatment with the BM extracts. Table 2 showed the percentage uptake of ^{99m}Tc-CDM in the group of rats that was treated with BM extract and in the control group. The results also reveal an increased and significant uptake ($P < 0.05$) ^{99m}Tc-CDM in brain, liver, lung, intestines and urine after treatment with the BM extracts. Table 2 also showed significant decrease in the uptake of this radiopharmaceutical in the heart, kidney, muscle, and spleen after treatment with the BM extracts.

4 Discussion

There is considerable evidence that the pharmacokinetics of radiopharmaceuticals may be altered by a variety of drugs (natural/synthetic), disease states, and surgical procedures. If unknown, such alterations may lead to poor organ visualization, a requirement to repeat the procedure, unnecessary radiation exposure, or even misdiagnosis (Mattos, 2000). It is worthwhile to establish the effects of various drugs (natural/synthetic) on the biodistribution of radiopharmaceuticals. As BM extract is a good antioxidant and have cognitive function on human memory (Roodenrys, 2002) this effect can explain the alteration of the radiopharmaceuticals biodistribution in brain and different organs. Gomes *et al* (Gomes, 1998) reported that mitomycin-C increased the uptake of ^{99m}Tc-MDP, ^{99m}Tc-DTPA in heart, stomach, kidneys, spleen and lung. A similar type of alteration effects was also observed following BM extract in the brain, heart, liver, lung, spleen, muscle, kidney, intestines and urine with ^{99m}Tc-ECD and ^{99m}Tc-CDM.

Some studies are available about the effects of several natural and synthetic substances on the biodistribution of the ^{99m}Tc radiopharmaceuticals. It was also reported that vincristine increases the uptake of ^{99m}Tc-MDP, ^{99m}Tc-DTPA, ^{99m}Tc-DMSA in heart, lungs, kidney, spleen, liver. Natural product such as Ginkgo Biloba extracts and chayote extracts can also induce the biodistribution of ^{99m}Tc labeled radiopharmaceuticals (Moreno, 2005, 2007; Dire, 2003). A similar alteration of biodistribution of BM extract treated rat organs (brain, heart, liver, lung, spleen, muscle, kidney, intestines and urine) with ^{99m}Tc-ECD and ^{99m}Tc-CDM radiopharmaceuticals were observed with respect to control group of rat organs.

Table 1. Effect of BM extracts on the biodistribution of ^{99m}Tc-ECD in rat

Organ	%ATI/g of organ ($n = 5$)	
	Control	BM extracts (100 mg/ml)
Brain	0.90 ± 0.05	1.52 ± 0.04
Heart	5.30 ± 0.33	4.20 ± 0.30
Liver	7.41 ± 0.61	9.30 ± 0.53
Lung	10.39 ± 1.05	11.44 ± 0.86
Spleen	6.50 ± 0.45	5.52 ± 0.90
Muscle	0.89 ± 0.31	0.66 ± 0.76
Kidney	4.97 ± 0.73	3.71 ± 0.91
Intestines	3.68 ± 1.60	5.38 ± 1.65
Urine	1.33 ± 0.46	4.82 ± 0.68

Table 2. Effect of BM extracts on the biodistribution of ^{99m}Tc-CDM in rat

Organ	%ATI/g of organ ($n = 5$)	
	Control	BM extracts (100 mg/ml)
Brain	0.27 ± 0.03	0.47 ± 0.07
Heart	3.15 ± 0.18	2.04 ± 0.30
Liver	3.94 ± 0.12	4.61 ± 0.22
Lung	5.68 ± 0.04	6.02 ± 0.19
Spleen	4.90 ± 0.26	4.12 ± 0.33
Muscle	0.50 ± 0.27	0.33 ± 0.06
Kidney	6.83 ± 0.41	6.08 ± 0.20
Intestines	0.69 ± 0.31	1.05 ± 0.44
Urine	0.35 ± 0.17	0.77 ± 0.30

5 Conclusions

In conclusion, the experimental model employed in

this study may a suitable method for evaluating pharmacokinetic interactions with BM extract. The results obtained suggest that this BM extract can act on the biodistribution of ^{99m}Tc-ECD and ^{99m}Tc-CDM in specific organs. The present study indicates that the medicinal plant extract and radiobiocomplex interaction depends on the experimental conditions employed and on the radiobiocomplex studied.

When the drug (natural plant extract/synthetic) interactions with radiobiocomplexes are unknown, the examination is not recommended, since the consequences of the procedure are the possibility of misdiagnosis and/or repetition of the examination, with an increase in the radiation dose administered to the patient. When the drug interactions with radiobiocomplexes are known, whether desirable or undesirable, the natural consequence is a correct diagnosis.

In the present study the uptake of ^{99m}Tc-ECD and ^{99m}Tc-CDM was increased in case of brain, liver, lung, intestines, and urine, and decreased in case of heart, spleen, muscle and kidney compared WITH that of control group of rats, by the BM extract therapy. The results could be explained by the therapeutic or immunosuppressive action of the studied medicinal plant extracts. Studies of the effects of BM extract on the biodistribution with other ^{99m}Tc-radiopharmaceuticals are now in progress. Although these alteration of the radioactivity of ^{99m}Tc-ECD and ^{99m}Tc-CDM were obtained by BM extracts in case of animals, caution is advisable in the interpretation of the nuclear medicine examination when the patient is using this herb. The knowledge about this phenomenon represents important clinical information for the best therapeutic decision and correct diagnosis.

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