BASIC RESEARCH

An experimental model to study the effects of a senna extract on the blood constituent labeling and biodistribution of a radiopharmaceutical in rats

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ABSTRACT Cassia angustifolia Vahl (senna) is a natural product that contains sennosides, which are active components that affect the intestinal tract and induce diarrhea. Authors have shown that senna produces DNA (deoxyribonucleic acid) lesions in Escherichia coli cultures and can act as an antifungal agent. Natural drugs can alter the labeling of blood constituents with technetium-99m (⁹⁹mTc) and can affect the biodistribution of radio-pharmaceuticals. In this work, we have evaluated the influence of a senna extract on the radiolabeling of blood constituents and on the biodistribution of the radiopharmaceutical sodium pertechnetate (Na⁹⁹mTcO₄) in Wistar rats. Twelve animals were treated with senna extract for 7 days. Blood samples were withdrawn from the animals and the radiolabeling procedure was carried out. The senna extract did not modify the radiolabeling of the blood constituents. A biodistributional assay was performed by administrating Na⁹⁹mTcO₄ and determining its activity in different organs and in blood. The senna extract altered the biodistribution of Na⁹⁹mTcO₄ in the thyroid, liver, pancreas, lungs and blood. These results are associated with properties of the chemical substances present in the aqueous senna extract. Although these assays were performed in animals, our findings suggest that caution should be exercised when nuclear medicine examinations using Na⁹⁹mTcO₄ are conducted in patients who are using senna extract.

KEYWORDS: Cassia angustifolia Vahl; Biodistribution; Radiolabeling; Rats; Technetium-99m.

Received for publication on September 17, 2010; First review completed on October 26, 2010; Accepted for publication on November 26, 2010

INTRODUCTION

Cassia angustifolia Vahl (senna) is a plant that belongs to the Fabaceae family. This branching shrub, which is found in abundance throughout South India, can grow up to 1.8 m in height. Extracts of this plant are used in folk medicine to treat certain gastrointestinal disorders.¹,² Hydroxanthracene glycosides, also known as senna sennosides, have been reported to stimulate the peristalsis of the colon and alter colonic absorption and secretion, which results in fluid accumulation and expulsion.³ Researchers² have suggested that a normal daily dose of senna in adults consists of two tablets with a sennoside content of 18 mg per 90 mg tablet. Other investigations⁴ have shown that sennosides induce diarrhea through changes in the intestinal tract. The laxative effect of this natural product has been linked to its content of anthraquinone glycosides.⁵ The antifungal activity⁶ of senna has been demonstrated previously and linked to a triterpenoid glycoside present in the butanolic seed extracts of senna. In addition, a study has demonstrated that an aqueous extract of senna can produce DNA (deoxyribonucleic acid) lesions but cannot induce cytotoxic or mutagenic effects in Escherichia coli cultures; the senna extract has also exhibited an antioxidant/antimutagenic effect in Escherichia coli cultures.⁷

Nuclear medicine images have allowed health professionals to measure physiological processes and identify changes related to various diseases. Disease can alter the biodistribution of a radiopharmaceutical, and the analysis of scintigraphic images can help physicians identify altered biological activity and diagnose clinical disorders.⁸,⁹ However, other factors such as drug interactions (with natural or synthetic compounds)¹²,¹³ can alter the biodistribution of a radiopharmaceutical.¹⁰,¹¹ If these drug interactions are not anticipated, poor image quality in the nuclear medicine examinations could lead to a misdiagnosis with a possible need to repeat the examination, thereby increasing the radiation exposure to the patient and the staff.¹¹,¹⁴
Molecular and cellular structures labeled with technetium-99m ($^{99m}$Tc) have been used in scintigraphy and single photon emission computed tomography (SPECT) procedures to label radiopharmaceuticals. Blood constituents labeled with $^{99m}$Tc have been used for imaging applications in the cardiovascular system, to detect gastrointestinal hemorrhage and to locate intramuscular hemangioma.$^9,15$

Natural products are widely used around the world for a variety of medical and domestic applications. However, some of the biological effects and biochemical properties of these products are not yet completely understood. Thus, experimental models can be used to improve our understanding of the cellular and systemic mechanisms of action and biological effects of these natural products. The aim of this work is to evaluate the effects of a senna extract on the labeling of blood constituents with $^{99m}$Tc and on the biodistribution of the radiopharmaceutical sodium pertechnetate in Wistar rats.

**MATERIALS AND METHODS**

The experimental models used 12 male Wistar rats (weight $309 \pm 26$ g) obtained from Laboratório de Cirurgia Experimental, Universidade do Estado do Rio de Janeiro (UERJ), Brazil. The Committee on Animal Research of the UERJ approved the protocols used in this work (CEA/121/2006). The animals were maintained under normal environmental conditions ($22 \pm 5^\circ$C, 12 h of light/dark cycle) with water and a normal diet. The animals were divided into treated ($n = 6$) and control ($n = 6$) groups. An extract prepared from a commercial sample of Cassia angustifolia Vahl (Laboratory of Luca de Maio, Brazil) was administered to the rats in the treated group. All of the experiments were carried out before the expiration date of this product.

The aqueous extract was freshly prepared using 400 mg of the herb and 10 mL of 0.9% NaCl. The solution was mixed in a vortex for 2 minutes and centrifuged (1500 rpm, 5 min, clinical centrifuge). The supernatant was approximately 40 mg/mL.

The supernatant solution was intragastrically administered (48 mg/kg/day) to the treated rats for 7 days. The control group received a saline solution (0.9% NaCl) in an identical manner.$^16$ The rats were observed daily to verify possible toxic effects.

To assess the effect of the senna extract on the labeling of blood constituents, samples of heparinized blood (0.5 mL) were collected from all animals on the seventh day and incubated with 0.5 mL of stannous chloride (1.2 µg/mL as SnCl$_2\cdot$H$_2$O; Sigma Chemical Co., USA) for 1 hour, as previously reported.$^17$ After this period of time, $^{99m}$Tc was added. The samples were centrifuged, and plasma (P) and blood cells (BC) were separated. Other aliquots of P and BC were also precipitated with trichloroacetic acid, and the soluble (SF) and insoluble (IF) fractions were separated. The radioactivity of the P, BC, IF-P, SF-P, IF-BC, and SF-BC was counted using a well gamma-counter (Packard Instrument Company, mod C5002, USA). The percentage of radioactivity (%ATI) was calculated using a previously reported procedure.$^{13}$

To evaluate the effect of the senna extract on the biodistribution of the radiopharmaceutical, 0.3 mL of the Na$^{99m}$TcO$_4$ radiopharmaceutical (3.7 MBq) was administered by the ocular plexus on the seventh day, as previously reported.$^{16}$ After 10 min, the animals were sacrificed, samples of blood were collected and the various organs (pancreas, thyroid, brain, testis, spleen, kidney, heart, stomach, lungs, liver, duodenum, large intestine, muscle and bone) were isolated. The mass of the organs was measured, and the $^{99m}$Tc radioactivity of each organ was determined using a well gamma-counter (Packard Instrument Company, mod C5002, USA). Samples of blood with a volume of 1 mL were considered to weigh 1 g. The percentage of radioactivity per gram of each organ (%ATI/gram) was calculated as described previously.$^{16}$

All data were presented as mean ± standard deviation, and the statistical analysis of the results was performed using an unpaired t-test. The level of statistical significance was set at $p<0.05$. InStat GraphPad software was used to perform the statistical analysis (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, USA).

**RESULTS**

The senna extract dose used in this study (i.e., 48 mg/kg/day) showed no laxative or toxic effects in the rats.

Table I shows the effect of the Cassia angustifolia Vahl aqueous extract on the distribution of radioactivity in blood cells and plasma constituents and in the insoluble and soluble fractions isolated from blood cells and plasma samples. The results indicate that the extract did not alter the distribution of $^{99m}$Tc in the blood constituents. Moreover, the fixation of the radionuclide in the insoluble fraction of plasma and bold cells samples is not also altered.

Table II shows the effect of the aqueous extract of Cassia angustifolia Vahl on the biodistribution of Na$^{99m}$TcO$_4$ (%ATI/g) in Wistar rats that either received (treated group) or did not receive (control group) the extract. The data in this table indicate that the senna extract significantly altered ($p<0.05$) the % ATI/gram of the Na$^{99m}$TcO$_4$ radiopharmaceutical in the thyroid (from 5.64 ± 2.27 to 3.16 ± 1.50), liver (from 0.79 ± 0.08 to 0.60 ± 0.12), pancreas (from 0.62 ± 0.23 to 0.39 ± 0.09), lungs (from 0.89 ± 0.14 to 0.69 ± 0.10) and blood (from 1.37 ± 0.23 to 0.90 ± 0.24).

| Compartment   | Control group (%ATI) | Treated group (%ATI) |
|---------------|----------------------|----------------------|
| BC            | 93.18 ± 1.94         | 92.17 ± 0.90         |
| P             | 6.82 ± 1.94          | 7.83 ± 0.90          |
| IF-P          | 72.98 ± 2.73         | 68.84 ± 4.14         |
| SF-P          | 27.02 ± 2.73         | 31.16 ± 4.14         |
| IF-BC         | 78.44 ± 2.09         | 76.08 ± 5.94         |
| SF-BC         | 21.56 ± 2.09         | 23.92 ± 5.94         |

Samples of blood from Wistar rats (treated and control) were incubated with stannous chloride and $^{99m}$Tc was added. The samples were centrifuged, and plasma (P) and blood cells (BC) were separated. Other aliquots of P and BC were precipitated with trichloroacetic acid, and soluble (SF) and insoluble (IF) fractions were also separated and counted. The radioactivity was counted and the percentage of radioactivity (ATI%) was calculated.
Table II - The effect of the senna extract on the biodistribution of Na\(^{99m}\)TcO\(_4\) in Wistar rats.

| Organ       | Control (%ATI/g) | Treated (%ATI/g) |
|-------------|------------------|-----------------|
| Blood       | 1.37 ± 0.23      | 0.90 ± 0.24**   |
| Bone        | 0.39 ± 0.13      | 0.31 ± 0.10     |
| Brain       | 0.07 ± 0.02      | 0.06 ± 0.02     |
| Duodenum    | 1.01 ± 0.26      | 0.81 ± 0.29     |
| Heart       | 0.48 ± 0.14      | 0.38 ± 0.15     |
| Kidney      | 0.66 ± 0.15      | 0.65 ± 0.12     |
| Large intestine | 0.40 ± 0.16       | 0.39 ± 0.06     |
| Liver       | 0.79 ± 0.13      | 0.69 ± 0.12*    |
| Lungs       | 0.89 ± 0.14      | 0.69 ± 0.10*    |
| Muscle      | 0.17 ± 0.02      | 0.16 ± 0.02     |
| Pancreas    | 0.62 ± 0.23      | 0.39 ± 0.09*    |
| Spleen      | 0.51 ± 0.11      | 0.47 ± 0.09     |
| Stomach     | 2.44 ± 1.08      | 2.19 ± 1.10     |
| Testes      | 0.20 ± 0.04      | 0.19 ± 0.05     |
| Thyroid     | 5.64 ± 2.27      | 3.16 ± 1.50*    |

Male Wistar rats were treated with an aqueous senna extract that was intragastrically administered daily. After seven days, Na\(^{99m}\)TcO\(_4\) (3.7 MBq) was administered and the animals were sacrificed. The animals’ organs were isolated, the mass of each organ was determined and the percentage of radioactivity per gram of each organ (%ATI/gram) was calculated (1 mL of blood was considered to weigh 1 g). The dose used in our work was 48 mg/kg/day and did not alter the biodistribution of blood constituents with \(^{99m}\)TcO\(_4\) was almost completely eliminated. The extract of Cassia angustifolia used in the present study (Table I) did not alter the biodistribution of the blood constituents, most likely because the generated metabolites did not exhibit oxidant properties. In this case, the reducing agent (stannous chloride) would not be oxidized by the metabolites of the Cassia angustifolia and the biodistribution efficiency would be not altered. This phenomenon is also most likely associated with the findings obtained with the Ginkgo biloba extract.29

The biodistribution assay is another experimental model used to evaluate the interactions between radiopharmaceuticals and drugs. Biodistribution is related to the distribution, uptake, retention and elimination of radiopharmaceuticals and depends on several factors including regional blood flow tissue metabolism and binding to the blood constituents.8 Altered biological behavior may also occur due to disease or interference caused by the pharmacodynamic effects of synthetic and natural drugs. An unknown interaction with radiopharmaceuticals can lead to a misdiagnosis along with a possible need to repeat the examination.9 The radiopharmaceutical sodium pertechnetate is generally distributed throughout the vasculature and interstitial fluid and is concentrated in the stomach, intestinal tract, thyroid and salivary glands.12,13 A biodistribution study has demonstrated an increase in the uptake of Na\(^{99m}\)TcO\(_4\) in the liver10 due to eggplant extract and an altered uptake in the duodenum, spleen, pancreas, stomach and blood10 due to Passiflora edulis flavicarpa extract. However, cauliflower extract does not alter the biodistribution of Na\(^{99m}\)TcO\(_4\) in mice.28 In the present study (Table II), the aqueous senna extract decreased the uptake of the Na\(^{99m}\)TcO\(_4\) radiopharmaceutical in the thyroid, liver, pancreas, lungs and blood. The action of the senna extract could generate metabolites capable of promoting morphological and physiological modifications in these organs and altering the biodistribution of Na\(^{99m}\)TcO\(_4\) in the treated animals. The effects of the senna extract on the gastrointestinal tract have been previously characterized,3,31,32 but its effects on other organs have not yet been completely established. In this work (Table II), no alteration of the gastrointestinal uptake of the Na\(^{99m}\)TcO\(_4\) radiopharmaceutical was found after treatment with the senna extract. These results indicate that no physiological changes would be observed in the animal’s gastrointestinal tissues due to treatment with the senna extract at the concentration used. No pathological changes were observed in these tissues under light microscopy examination at 3 hours and at 3, 4 and 6 weeks after the gastric administration of the senna extract.32 However, the senna aqueous extract decreased the uptake of the Na\(^{99m}\)TcO\(_4\) radiopharmaceutical in the thyroid, liver, pancreas, lungs and blood. The action of the...
senna extract generates metabolites that alter the biodistribution of Na\textsuperscript{99m}TcO\textsubscript{4} in the treated animals and promotes enteric motor activity.\textsuperscript{33} The effect of the Cassia angustifolia extract on the thyroid, pancreas, lungs and blood is unclear. However, the decreased uptake of Na\textsuperscript{99m}TcO\textsubscript{4} in the liver could be due to the anti-hepatoprotective activity of the Cassia angustifolia extract; such activity has been described in studies with human liver cancer cell lines.\textsuperscript{34}

The two experimental models used in this study suggest that substances in the aqueous senna extract would not alter the labeling of blood constituents with Na\textsuperscript{99m}Tc but would alter the uptake of sodium pertechnetate in some organs. Although these assays were performed in animals, the findings suggest that caution should be exercised while interpreting the results of Na\textsuperscript{99m}TcO\textsubscript{4}-based nuclear medicine examinations in patients using senna extract. Moreover, our findings reinforce the importance of experimental models that use a radiouclide to evaluate biochemical properties and to study the biological effects associated with synthetic and natural drugs.

ACKNOWLEDGEMENTS

This research was supported by Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Universidade do Estado do Rio de Janeiro (UERJ), Conselho Nacional de Pesquisa e Desenvolvimento (CNPq) and Instituto Nacional do Câncer (INCA).

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