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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

Acetylsalicylic Acid and Labeling of Blood Constituents with Technetium-99m

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ABSTRACT

Acetylsalicylic acid is the drug most used an anti-inflammatory agent and for secondary prevention of thrombotic phenomenon. Drugs can modify the labeling of blood constituents with technetium-99m (99mTc). The aim of this work was to evaluate the effect of in vitro or in vivo assays with acetylsalicylic acid on the labeling of the blood constituents with 99mTc. In vitro assay was performed with samples of whole blood from Wistar rats incubated with acetylsalicylic acid (1.0 mg/ml) for one hour before the 99mTc-labeling process. For in vivo assay, Wistar rats were treated with acetylsalicylic acid (1.5 mg/kg) during one hour, and the whole blood was withdrawn for the 99mTc-labeling process. Saline was used in control groups. Data showed that the fixation of 99mTc to the blood constituents was not significantly ($p>0.05$) modified in in vitro and in vivo assays with acetylsalicylic acid, at least not when the experiments were carried out with the doses normally used in human beings.

Key words: Acetylsalicylic Acid; Blood Constituents; Technetium-99m

INTRODUCTION

Acetylsalicylic acid is the most widely used drug for antipyretic, analgesic, anti-inflammatory action and for the secondary prevention of ischemic events in the brain, heart and peripheral circulation (Ruth and Calverley, 1994; Antithrombotic Trialists' Collaboration, 2002; De La Cruz et al., 2004). Its antipyretic, analgesic and anti-inflammatory actions are based on irreversible inhibition of the cyclooxygenase enzyme that is responsible for the prostaglandin synthesis and some autacoids (Catella-Lawson, 2001; Amann and Peskar, 2002; Aude and Mehta, 2002). The acetylsalicylic acid effects on thrombotic phenomenon seem to be mainly a result of its antiplatelet action (Grotta et al., 1985; Catella-Lawson, 2001).

It has been postulated that the ability of acetylsalicylic acid to prevent cerebrovascular accidents is related to the inhibition of oxidative stress (Sagone et al., 1987; Guerrero et al., 2004). In this context, it was demonstrated that acetylsalicylic acid reduces hypercholesterolemic atherosclerosis by decreasing the generation of oxyradicals (Prasad et al., 2003). However, acetylsalicylic acid can cause adverse gastrointestinal effects such as gastric ulceration, erosive gastritis, gastrointestinal hemorrhage and exacerbation of peptic ulcer symptoms at therapeutic doses (Bollini et al., 1992; Langman et al., 1994). In addition, other adverse effects related

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to pH and ion balance such as respiratory alkalosis with increased Na^+, K^+ and bicarbonate excretion (Lauwerys and Bernard, 1989; Nuyts et al., 1989) can be observed when analgesic or anti-inflammatory doses of acetylsalicylic acid are used.

Red blood cells labeled with technetium-99m (99mTc-RBC) have been used in nuclear medicine in a number of evaluation procedures for the gastrointestinal bleeding sites (Wong et al., 2004; Zaman et al., 2004; Olds et al., 2005), peripheral arterial blood flow (Harel et al., 2005), hepatic hemangiomas (Artiko et al., 2004; Verdu et al., 2005), renal carcinoma (Cortes et al., 2003) and splenic reticuloendothelial system (Jin et al., 2004; Slart et al., 2004).

Data have shown that some drugs can modify the labeling of blood constituents, such as the RBC, with 99mTc and should alter the results obtained in the daily routine procedures in nuclear medical laboratories (Hladik III et al., 1987; Capriles et al., 2002; de Oliveira et al., 2002; Frydman et al., 2004; Fonseca et al., 2005). The aim of this work was to investigate the *in vitro* and *in vivo* effects of acetylsalicylic acid on the labeling of blood constituents with 99mTc.

MATERIALS AND METHODS

Animals

Adult male Wistar naive rats (3-4 months of age, body weight 250-350g) were housed, five per cage, in an environment controlled room with light/dark cycle conditions (12 hours light/12 hours dark; lights on at 6:00 a.m.). Animals had free access to water and food and ambient temperature was kept at 25 ± 2°C. Experiments were conducted in accordance with the Department Committee of Animal Care.

Drugs

Acetylsalicylic acid used in this study was purchased from Bayer (Brazil).

In vitro assay with acetylsalicylic acid

Heparinized samples (0.5 ml, n = 6) of whole blood were incubated with 100 µl of saline acetylsalicylic acid solution at 1.0 mg/ml for 1 hour at room temperature before the 99mTc labeling procedure. Samples of whole blood (0.5 ml, n = 6) incubated with saline solution (NaCl 0.9%) were used as the control group.

In vivo assay with acetylsalicylic acid

The animals (n = 5) were treated by gavage during 1 hour with 1.5 mg/kg acetylsalicylic acid dose, and, heparinized whole blood was withdrawn for 99mTc labeling procedure. Animals (n = 5) treated with saline solution were used as control group.

99mTc labeling of blood constituents

After *in vitro* or *in vivo* treatment with acetylsalicylic acid, the samples (0.5 ml) of whole blood were incubated with stannous chloride (1.2 µg/ml) (Sigma Chemical Co., St Louis, USA) during 1 hour. After this period, 99mTc (100 µl, 37 MBq/ml), as sodium pertechnetate, recently milked from a molybdenium-99/technetium-99m generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, Brazil), was added and the incubation continued for another 10 minutes. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20µl) of P and BC were precipitated in 1.0 ml of trichloroacetic acid (5%), and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in P, BC, IF-P, SF-P, IF-BC and SF-BC were determined in a gamma counter (Auto-gamma, Packard Instrument Company, Illinois, USA). The percentage of radioactivity (%ATI) was calculated, as previously described (Bernardo-Filho et al., 1994).

Statistical analysis

Data are reported as means ± SD of %ATI, and the treated and control groups were compared by the Student *t* test with a significance level $p<0.05$. InStat Graphpad software was used to perform statistical analysis (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California, USA).

RESULTS

The %ATI in P, BC, IF-P, SF-P, IF-BC and SF-BC from whole blood treated *in vitro* with acetylsalicylic acid (1.0mg/ml) are shown in Fig. 1. The data presented in this figure show that, in this *in vitro* assay with the drug at the concentration used, small, but not significant

($p>0.05$), alterations in the uptake of 99mTc by plasma, blood cells and insoluble and soluble fractions of plasma and blood cells were found.

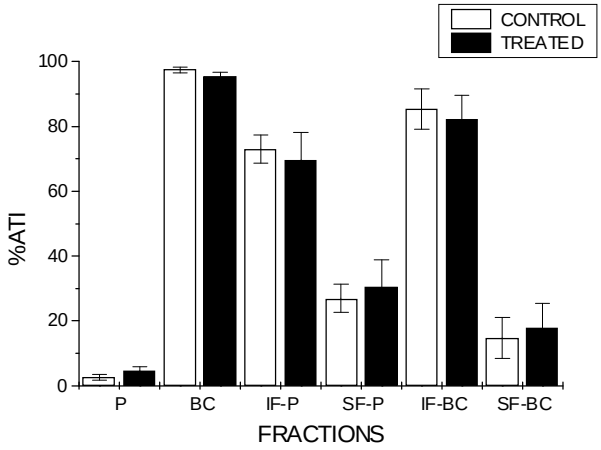

Figure 1 - %ATI in different fractions of blood constituents in *in vitro* assay with acetylsalicylic acid. Heparinized samples (0.5 ml, n = 6) of whole blood were incubated with 100 µl of acetylsalicylic acid solution (1.0 mg/ml) during 1 hour at room temperature. After this period, the labeling procedure was performed and %ATI was calculated. Whole blood incubated with saline solution constituted the control group (n = 6). Plasma (P), blood cells (BC), insoluble fraction of plasma (IF-SF), soluble fraction of plasma (SF-P), insoluble fraction of blood cells (IF-BC) and soluble fraction of blood cells (SF-BC).

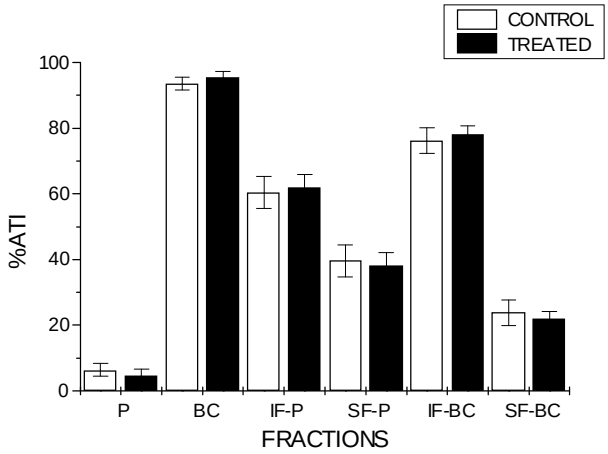

Figure 2 - %ATI in different fractions of blood constituents in *in vivo* assay with acetylsalicylic acid. The animals (n = 5) were treated with a 1.5 mg/kg dose of acetylsalicylic acid during 1 hour. Heparinized whole blood was withdrawn, the labeling procedure was performed, and the %ATI was calculated. Animals treated with saline solution (n = 5) constituted the control group Plasma (P), blood cells (BC), insoluble fraction of plasma (IF-SF), soluble fraction of plasma (SF-P), insoluble fraction of blood cells (IF-BC) and soluble fraction of blood cells (SF-BC).

The %ATI in P, BC, IF-P, SF-P, IF-BC and SF-BC from whole blood of animals treated *in vivo* during 1 hour with acetylsalicylic acid (1.5 mg/kg) can be seen in Fig.2. The data presented in this figure indicate that the *in vivo* treatment with this drug at the concentration used did not significantly ($p>0.05$) modify the uptake of 99mTc by plasma, blood cells and insoluble and soluble fractions of plasma and blood cells.

DISCUSSION

Therapeutic drugs can modify the nature or amount of the 99mTc-radiopharmaceutical bound to blood constituents and result in an unexpected behavior of the radiopharmaceutical (Hladik III et al., 1987; Hesslewood and Leung, 1994; Sobal and Sinzinger, 2001; Welling et al., 2002; Fonseca et al., 2005). Thus, the evaluation of the influence of drugs on the fixation of 99mTc in blood constituents is important. However, the data from these studies are relatively scarce, and the effects of pharmacologically active agents on the diagnostic by radiopharmaceuticals can be evaluated.

The data obtained in this work showed that acetylsalicylic acid, at a concentration similar to that used in anti-inflammatory therapy, as antipyretic or as an analgesic drug in humans, did not modify the fixation of 99mTc to the blood constituents of rats (Figs. 1 and 2).

Other data showed that acetylsalicylic acid can decrease the labeling efficiency of white blood cells (Ellis and Sampson, 1999) and that salicylate treatment may be interrupted one week before initiation of a thyroid uptake study with radioiodine (Hladik III et al., 1987).

The lowest effective plasma concentration of acetylsalicylic acid is about 150 μg/ml, and, at plasma concentrations as high as 350 μg/ml, toxic effects such as tinnitus and hyperventilation can occur (Chalasani et al., 1996; Porto Arceo, 2003). Thus, the results obtained in this work showed that acetylsalicylic acid in the plasma concentrations found in humans should not alter the uptake of the 99mTc by the blood constituents studied.

In the 99mTc-RBC labeling procedure, stannous ion (Sn^{+2}) is used as the reducing agent, so compounds or conditions that interfere with its action can alter the fixation of 99mTc on these constituents (Hladik III et al., 1987; Bernardo-Filho et al., 1994).

Several effects of acetylsalicylic acid have been associated with its antioxidant properties (Steer et al., 1997; Wu et al., 2002). However, other authors have shown that, when this drug is given orally, its main metabolite is salicylic acid, which presents an antioxidant effect higher than that of acetylsalicylic acid (Guerrero et al., 2004). Thus, under the conditions used in this study, the absence of an effect of *in vitro* or *in vivo* acetylsalicylic acid treatment on uptake of 99mTc by blood constituents may be related to the small antioxidant effect of this drug on stannous ion. In addition, the absence of alterations in 99mTc fixation in blood constituents may be influenced by the fact that the half-life of salicylic acid in human plasma is about 15 minutes (Needs and Brook, 1985), and, in this study, a higher treatment period (1 hour) was used in rats.

In conclusion, the data presented in this work showed that *in vitro* and *in vivo* treatment with acetylsalicylic acid at the concentrations usually found in the plasma of human beings did not modify the labeling of blood constituents with 99mTc. Although the experiments were carried out with rats, it is possible to suggest that acetylsalicylic acid at therapeutic doses should not interfere with the procedure in nuclear medicine involving the labeling of blood constituents with 99mTc.

RESUMO

Ácido acetilsalicílico é a droga mais usada como antiinflamatório e para prevenção de fenômenos trombóticos. Drogas podem modificar a marcação de constituintes sangüíneos com tecnécio-99m (99mTc). O objetivo deste trabalho foi avaliar o efeito do ácido acetilsalicílico *in vitro* ou *in vivo* na marcação dos constituintes sangüíneos com 99mTc. Ensaios *in vitro* foram realizados com amostras de sangue total de ratos Wistar incubadas com ácido acetilsalicílico (1.0mg/ml) 1 hora antes do processo de marcação com 99mTc. Para ensaios *in vivo*, ratos Wistar foram tratados com ácido acetilsalicílico (1.5mg/kg) durante 1 hora e, em seguida, o sangue total foi retirado para o processo de marcação com 99mTc. Salina foi usada nos grupos controles. Dados mostraram que nos ensaios *in vitro* e *in vivo* com ácido acetilsalicílico, a fixação do 99mTc nos

constituintes sangüíneos não foi significativamente (p>0.05) modificada, pelo menos, quando os experimentos foram realizados com doses normalmente usadas em humanos.

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