Environmental Toxicology and Pharmacology 63 (2018) 1–5

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Cytotoxic and genotoxic effects of antihypertensives distributed in Brazil by social programs: Are they safe?

Maria Fernanda de Moura Leão[a], Jonathaline Apollo Duarte[a], Patrícia Dutra Sauzen[b], Jacqueline da Costa Escobar Piccoli[a,b], Luís Flávio Souza de Oliveira[a,b], Michel Mansur Machado[a,b,*]

[a] Postgraduate Program in Pharmaceutical Sciences, Federal University of Pampa, BR 472, Km 585, Mail box 118, Uruguaiana, RS, CEP: 97500-970, Brazil
[b] Pharmacy Course, Federal University of PAMPA, BR 472, Km 585, Caixa postal 118, Uruguaiana, RS, CEP: 97500-970, Brazil

ARTICLE INFO

Keywords:
Anti-hypertensives
Genotoxicity
Macrophages
Lymphocytes
Cell culture

ABSTRACT

Hypertension, a chronic non-transmissible multifactorial condition, it is highly frequent in Brazil, affecting about 32.5% of the population over 25 years of age. It is characterized by the sustained increase in systolic and diastolic blood pressure levels above 140 mmHg and 90 mmHg, respectively. It is the major aggravating factor in cardiovascular complications and the appearance of other comorbidities. Aiming to promote greater adherence to treatment and improve the population's access to basic medicament, in 2004 the Federal Government created the *Programa Farmácia Popular do Brasil* (PFPB); partnership with private institutions that provides the population with medicament to control hypertension, free of charge or subsidized at up to 90% of the value. The PFPB distributes the anti-hypertensives atenolol, captopril, enalapril, hydrochlorothiazide, losartan and propranolol. In this way, this work aims to evaluate the genotoxic potential of antihypertensives in human lymphocytes and macrophages, since they are widely used drugs and with few studies about their genotoxicological safety. The tests were developed from cell cultures treated with five different antihypertensive concentrations, all based on plasma peaks, evaluating cell viability, DNA damage index and DNA double strand breakdown. The results show that, as the concentration of captopril and enalapril maleate increased, cell viability decreased. In addition, a DNA damage was observed with the use Captopril and Enalapril in the higher concentrations. Hydrochlorothiazide also caused DNA damage in the five doses tested. Regarding the breaking of double strands of DNA, all the compounds showed increased ruptures. This decrease in dsDNA is dose dependent for all compounds tested. The set of results shows that the use although frequent still requires care and greater knowledge. In general, the antihypertensive drugs that proved to be safer in relation to the genetic damage tested were Losartan and Propranolol.

1. Introduction

Arterial Hypertension (HTA) is a clinical condition comprising multiple factors and is characterized by sustained increase in systolic and diastolic blood pressure above 140 mmHg and 90 mmHg, respectively (Paniz et al., 2010). In Brazil, HTA is the most frequent clinical condition among chronic non-communicable diseases, affecting approximately 32.5% of the population older than 25 years; among the elderly, HTA is estimated to affect more than 60% of this population. Data from the World Health Organization (WHO) estimate that 40% of the world's population is affected by HTA, most of which are diagnosed in countries where the population's income is lower (BRASIL D. Disponível, 2013; Andrade et al., 2013). Hypertension is the most important risk factor for cardiovascular complications, since elevated blood pressure levels are associated with an increased risk of comorbidities, such as acute myocardial infarction, nephropathies, cerebrovascular and cerebral vascular accidents. HTA and its comorbidities have a high impact on the work and income of the affected population, estimated at US $ 4.18 billion between the years 2006 and 2015 (Gontijo et al., 2012; Borim et al., 2011).

Aiming to broaden the population's access to basic medicines, thus promoting adherence to treatment and its effectiveness, in addition to reducing the complications generated by hypertension and other chronic diseases, the federal government created in 2004 the *Programa Farmácia Popular do Brasil* (PFPB), and since its inception until 2007, has served approximately 93 million users (Geral and da reunião

* Corresponding author at: Universidade Federal do Pampa – Campus Uruguaiana, BR 472, Km 585, Caixa postal 118, Uruguaiana, RS, CEP: 97.500-970, Brazil.
E-mail address: michelmachado@unipampa.edu.br (M.M. Machado).

https://doi.org/10.1016/j.etap.2018.08.005
Received 14 February 2018; Received in revised form 3 August 2018; Accepted 8 August 2018
Available online 09 August 2018

Table 1
Concentrations of the drugs used in the tests.

Drug	PP/10 (ng/mL)	PP/2 (ng/mL)	Plasma peaks – PP [a] (ng/mL)	2xPP (ng/mL)	10xPP (ng/mL)	References
Atenolol	24.87	124.35	248.7	497.4	2.487	(Arnold et al., 2015)
Captopril	22.5	112.5	225	450	2.250	(Vogel et al., 2014)
Propranolol Hydrochloride	1.45	7.25	14.5	29	145	(Rouge et al., 1998)
Hydrochlorothiazide	14.2	71	142	284	1.420	(Chik et al., 2014)
Losartan	25.26	126.3	252.6	505.2	2.526	(Zhanga et al., 2009)
Enalapril Maleate	7.15	35.75	71.5	143	715	(Beermann and Groschinsky-Grind, 1977)

[a] The plasma peaks were chosen based on the medicines provided by the *Programa Farmácia Popular*: Atenolol 25 mg, Captopril 25 mg, Propranolol Hydrochloride 40 mg, Hydrochlorothiazide 25 mg, Losartan 50 mg and Enalapril Maleate 10 mg.

Plenária, 2018; Du Bocage Santos-Pinto et al., 2011). In 2006, the "*Aqui tem Farmácia Popular*" modality was created, based on the co-payment of the Ministry of Health in partnership with private pharmacies, offering drugs subsidized up to 90% for treatment of glaucoma, dyslipidemias, Parkinson's disease; and free medications for diabetes, asthma and hypertension treatments (Du Bocage Santos-Pinto et al., 2011; de Oliveira and Bárta, 2013). As antihypertensives, PFPB distributes the following medicines free of charge: atenolol, captopril, propranolol hydrochloride, hydrochlorothiazide, losartan and enalapril maleate (de Oliveira and Bárta, 2013).

In 2004, the National Health Surveillance Agency changed the registration rules for new medicines, following the proposal of international regulatory agencies, which included tests of genetic toxicity (Gava et al., 2010). The drugs distributed by the PFPB that are used in the treatment of hypertension, obtained their records before the year 2004, without the proof of the genotoxicological risks (Póvoa et al., 2014).

The topic of antihypertensive safety is not a new topic. Brambilla and Martelli (Brambilla and Martelli, 2006) published in 2006 a compendium of genotoxicity and carcinogenicity information of antihypertensive drugs. Data from 164 marketed drugs were collected. Of the 164 drugs, 65 (39.6%) had no retrievable genotoxicity or carcinogenicity data; this group was comprised largely of drugs marketed in a limited number of countries. The remaining 99 (60.4%) had at least one genotoxicity or carcinogenicity test result. Of these 99, 48 (48.5%) had at least one positive finding: 32 tested positive in at least one genotoxicity assay, 26 in at least one carcinogenicity assay, and 10 gave a positive result in both at least one genotoxicity assay and at least one carcinogenicity assay. This reality has been seen in other countries, and for other drugs as well, for some time, as shown by Snyder in his review (Snyder and Green, 2001). In this article, Snyder and Green surveyed drugs that had already been marketed and found that of 467 drugs for sale, 115 had no genotoxicity information at all, and of the other compounds, 101 had positivity in at least one pre-test indicator for parameters of genotoxicity.

In view of the improvement in the health of the users, the great use of antihypertensive drugs by the population and the fact that all the antihypertensives distributed by the PFPB obtained their registers before 2004, it becomes indispensable to the evaluation of the genotoxicological safety of drugs widely used in therapy.

2. Materials and methods

Drugs used in the tests: samples of the six antihypertensive drugs used in the tests, atenolol, captopril, enalapril, hydrochlorothiazide, losartan and propranolol, have external quality control analysis reports and purity greater than 99.5%.

2.1. Lymphocytes cultures

The lymphocytes cultures were prepared using 10 mL of venous blood taken from the medial cubital vein of a 23-year-old healthy male volunteer donor who had not consumed alcohol, smoked, or taken any medication that could interfere with the scientific results in the last 72 h. Blood was collected into a heparin-containing Vacutainer® (approved by the Research Ethics Committee of the Federal University of Pampa, n°. 27,045,614.0.0000.5323). Lymphocytes were isolated with Histopaque-1077® (Sigma-Aldrich, St. Louis, EUA) and transferred to the culture medium containing 9 mL of RPMI 1640 supplemented with 20% fetal bovine serum and 1% streptomycin / penicillin, as described in previous work (Machado et al., 2011; dos Santos Montagner et al., 2010). The cells were conditioned in culture flasks and placed in a microenvironment at 37 °C in 5% CO_2 environment for 72 h.

2.2. Macrophages culture

The isolation of macrophages from whole blood obtained above was performed by centrifugation and the density difference between the leukocyte cells using centrifugation methods and plastic adherence/monocyte glass as described in previous studies (Delirezh et al., 2013; Delirezh and Shojaeefar, 2012; Davis, 1992). Obtained cells of interest, activation of monocytes occurred using lipopolysaccharide (LPS), 1 µg/mL in RPMI 1640 supplemented with 1% penicillin/streptomycin, 25% fetal bovine serum at 37 ± 1 °C and atmosphere containing 5% CO_2 and 95% humidity for 2 h. This exposure result in the formation of macrophages derived from monocytes which are used in the studies (Arnold et al., 2015; Vogel et al., 2014).

Treatment of the cultures and selection of tests: All cultures received antihypertensive drug addition in the final volume of 1000 µL. All were diluted in pH 7.4 phosphate buffer. The groups tested were: Negative Control (NC) with phosphate buffer pH 7.4, Positive Control (PC) with Bleomycin 3 µM, Atenolol, Captopril, Propranolol, Hydrochlorothiazide, Losartan and Enalapril Maleate Group, all at the concentrations shown in Table 1 - Plasma Peak (PP), 2xPP, 10xPP, PP/2 and PP/10. All tests were performed in triplicate. Brazil follows the security assessment protocols proposed by the *Organization for Economic Cooperation and Development* (OECD). The tests performed here were selected and followed the indications of these protocols for their experimental design when applicable or the indications when suggested.

Effects of the Drugs on the cell viability of the analyzed cells: The analyzed parameter for evaluation of cytotoxicity was cell viability through the loss of membrane integrity using the trypan blue method (Burow et al., 1998). This requires put the sample in contact with the Trypan blue, which stains dead cells. The analysis was performed using an optical microscope at 400 × . One hundred cells were counted.

Quantification of double strands of DNA (dsDNA): For this assay, the Sigma® fluorescence DNA quantification kit consisting of: standard DNA solution, bisBenzimide solution H33258 and fluorescence test buffer was used. Initially, a calibration curve was prepared with the standard DNA solution. Subsequently, a 1æg / ml solution of BisBenzimide was prepared according to the manufacturer's instructions using the fluorescence buffer. One ml of this solution was added in a cuvette to read the blank value. With the cuvette still inside the fluorimeter, 50 µL of the sample was added and, after homogenization,