Life Sciences 141 (2015) 137–142

Contents lists available at ScienceDirect

Life Sciences

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

Study of the safety of methylphenidate: Focus on nephrotoxicity aspects

Luiza Herbene Macedo Soares Salviano [a], Maria Isabel Linhares [b], Klistenes Alves de Lima [d], Alana Gomes de Souza [d], Dânya Bandeira Lima [c], Antônio Rafael Coelho Jorge [e], Marcus Felipe Bezerra da Costa [c], Adriano José Maia Chaves Filho [e], Alice Maria Costa Martins [a,b,c,d], Helena Serra Azul Monteiro [b,e], Teresa Maria de Jesus Ponte Carvalho [a,d], Marta Maria de França Fonteles [a,b,c,d,*]

[a] Postgraduation Program in Development and Technological Innovation in Medicines (PPGDITM), Federal University of Ceará (UFC), Brazil
[b] Postgraduation Program in Pharmacology/UFC, Brazil
[c] Postgraduation Program in Pharmaceutical Sciences/UFC, Brazil
[d] Pharmacy Course/UFC, Brazil
[e] Department of Physiology and Pharmacology/UFC, Brazil

ARTICLE INFO

Article history:
Received 14 February 2015
Received in revised form 13 August 2015
Accepted 21 September 2015
Available online 25 September 2015

Keywords:
Methylphenidate
Nephrotoxicity
Renal perfusion

ABSTRACT

Aims: Methylphenidate (MPD) is increasingly prescribed for the treatment of Attention Deficit Hyperactivity Disorder and there are concerns about its appropriate use. Furthermore, little is known about the potential nephrotoxicity in patients using MPD. This study aimed to investigate the safety of MPD, with focus on the possible effects of this drug on renal function.
Main methods: We investigated the effects of MPD on renal perfusion system and renal tubular cells through *in vivo* and *in vitro* experimental models.
Key findings: In the *in vivo* experiments, 24 h and 48 h after MPD administration, urea, creatinine, creatinine clearance, and the fractional excretion of sodium and potassium were not changed. In the isolated kidney perfusion, MPD significantly reduced urinary flow, glomerular filtration rate and the percentage of tubular sodium transport. However, the perfusion pressure, renal vascular resistance and the percentage of tubular potassium transport were unchanged in this system. In the canine renal epithelial cell line MDCK culture, MPD was not cytotoxic and, in histopathological analysis, MPD did not promote alterations.
Significance: Our findings suggest a possible nephrotoxic effect of MPD, since it altered renal function by reducing the glomerular activity, urinary flow and sodium transport. These effects need to be further investigated in order to minimize potential harms associated with the use of MPD.

1. Introduction

Methylphenidate (MPD) is the drug of first choice for the treatment of Attention Deficit Hyperactivity Disorder (ADHD). The MPD is the most consumed psychostimulant in the world [16]. Its production rose from 2.8 tons in 1990 to nearly 38 tons in 2006; this is not only due to its link to ADHD, but mainly due to intense publicity about the drug [16]. In Brazil, in line with this global trend, consumption has also grown in each year. In 2007, nearly 1.15 million boxes of MPD were bought in this country [15].

There is widespread concern about the pharmaceutical industry's aggressive marketing of MPD, which only highlights the potential benefits of the drug, thus increasing possibility of inappropriate and unsafe use. These advertisements may also lead to abusive consumption of MPD due to its putative effect of enhancement of cognitive function (one of the reasons young university students reported for using it), in addition to inappropriate use in misdiagnosed ADHD [29].

The diagnosis of ADHD is clinical and there are no objective measurements to confirm diagnosis [19,39]. This situation allows that ADHD may be overdiagnosed, leading to an increase in the prescription of MPD [19,29,39]. Methylphenidate is indicated for ADHD for children from six years of age, adolescents and adults; however, its 'unapproved' use in children under six years has also been increasing [12].

MPD is completely and rapidly absorbed when administered orally, and it readily crosses the blood–brain barrier [8,40]. This drug is metabolized primarily through deesterification in the liver, which generates the main metabolite ritalinic acid, which is excreted renally. The half-life of MPD is approximately 2 h after absorption, and the half-life of its metabolites is about 7 h [8]. The pharmacokinetic of MPD in children is essentially the same as for adults, and the bioavailability in humans is similar to that observed in rats and monkeys [40].

Although methylphenidate has a satisfactory safety profile [1,28], several adverse reactions have been reported including headaches,

* Corresponding author at: Department of Pharmacy, Faculty of Dentistry, Nursing and Pharmacy, Federal University of Ceará, Street Capitão Francisco Pedro, 1210, CEP: 60430-375, Fortaleza, Ceará, Brazil.
E-mail address: martafonteles@yahoo.com.br (M.M. de França Fonteles).

http://dx.doi.org/10.1016/j.lfs.2015.09.014
0024-3205/

loss of appetite, weight loss, insomnia, abdominal pain and growth retardation. Other effects include dependency, irritability in ADHD patients, worsening of hyperactivity symptoms, nausea, tachycardia, increased anxiety, risk of abuse, and cardiovascular risks that may occur with chronic use of the drug [1].

Deaths from the abuse of methylphenidate have been reported [20,23,35]. In one case of a young adult, parenteral overdose of this drug resulted in multiple organ failure, with hepatic, renal, pancreatic, pulmonary and central nervous system complications. Of particular note in this patient were effects on renal function (poor urine output and rising levels of serum creatinine and urea) in addition to hypotension, tachypnea, tachycardia and abnormal blood gases [35].

Despite this, little is known about the effect on renal function in patients using MPD. Therefore, it is important to examine these aspects of its safety for its use by the general population [31].

The objective of this study was to investigate the safety of Ritalin® (methylphenidate *hydrochloride*) by *in vitro* and *in vivo* assays, with a particular focus on the possible effects of this drug on renal function.

2. Material and methods

2.1. Animals and drug used

The animals used in this study were adult male *Wistar* rats, weighing 250 to 300 g, from the Vivarium of the Department of Physiology and Pharmacology, Federal University of Ceará (UFC). The animals were kept in standard cages, at a controlled temperature (24 ± 2°C) with a 12-h dark/light cycle and food and water ad libitum. Twenty-four hours prior to the kidney perfusion experiments, the animals were put on a fast with free access to water. Studies were carried out in compliance with national and international laws and guidelines for the use of animals in biomedical research and under the consent and surveillance of Ethics Committee on Animal Research (Protocol number: 09/2012/UFC). Moreover, the experiments were performed to minimize the number of rats used and their suffering, following the ethical doctrine of the three "R"s — reduction, refinement and replacement.

The methylphenidate *hydrochloride* (Ritalin®) used in the experiment was purchased from its manufacturer (Novartis Laboratory) and dissolved in distilled water.

2.2. In vitro assays

2.2.1. Isolated kidney perfusion

The rats were fasted for 24 h with free access to water. The animals were anesthetized with sodium pentobarbital (50 mg/kg, ip) and after careful dissection of the right kidney, the right renal artery was cannulated via the mesenteric artery without interrupting the flow, as described by Bowman and Maack [2], Weiss [41] and Nishiitsutji-Uwo et al. [27]. The perfusion fluid (perfusate) consisted of a modified Krebs–Henseleit solution (MKHS) with the following composition (in mmol/L): 118 NaCl, 1.2 KCl, 1.18 KH_2PO_4, 1.18 $MgSO_4 \cdot 7H_2O$, 2.5 $CaCl_2 2H_2O$ and 25 $NaHCO_3$. Bovine serum albumin (BSA 6 g%; fraction V), urea (0.075 g), inulin (0.075 g) and glucose (0.15 g) were added to the solution, resulting in a final perfusate volume of 100 mL. The pH was adjusted to 7.4. There is a constant maintenance of dialyzable substances in the perfusate, and the oxygenation (95% O_2/5% CO_2) and temperature (37 °C) are adjusted to the system [25]. In each experiment, 100 mL of MKHS were recirculated for 120 min. The perfusion pressure (PP) was measured at the end of the stainless steel cannula in the renal artery. The inulin of the perfusate and of the urine was measured using direct hydrolysis, as in Walser et al. [38] and Fonteles et al. [9]. The photometric readings were carried out in a spectrophotometer and osmolarity was measured using an osmometer (*Vapor pressure osmometer* — Model 5520 ESCOR).

The experiments were started after stabilization of the organ to the new conditions. MPD (10 μg/mL) was added to the system at 30 min after the beginning of the renal perfusion. Every 5 min the perfusion pressure and perfusion flow were recorded for 120 min. Urine and perfusion fluid samples were collected every 10 min for determination of renal parameters: Urinary Flow (UF), Glomerular Filtration Rate (GFR), Perfusion Pressure (PP), Renal Vascular Resistance (RVR) and percentage of transported tubular sodium (%TNa^+) and potassium (%TK^+), in line with Martinez-Maldonado et al. [22], and Fonteles et al. [9]. The results were compared to the internal control group, at 30 min early in each experiment. The treatment group was also compared to the control group, in which the kidneys were perfused with MKHS alone.

The renal perfusion system used in our experiments was developed by Bowman and Maack [2] and Ross [33] and modified by Fonteles et al. [9] by adapting the Silastic artificial lung, based on the Hamilton et al. [13] model.

2.2.2. Cell culture

MDCK (*Madin–Darby Canine Kidney*) renal epithelial tubular cells were used. The *MDCK* cells were kindly donated by Chemistry Laboratory from Biochemistry Institute of São Paulo University—USP, São Paulo, Brazil. They were grown in cell culture flasks with RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin/streptomycin). Cells were maintained at 37°C in an atmosphere of 95% O_2/5% CO_2, and then exposed to trypsin–EDTA (0.25/0.02% *v*/*v*). Afterwards, the cells were suspended in culture medium with FBS and quantified in a Neubauer chamber. MDCK cells were plated at a concentration of 1×10^5 cells/mL in 96-well plates for 24 h, after which the experiments were initiated.

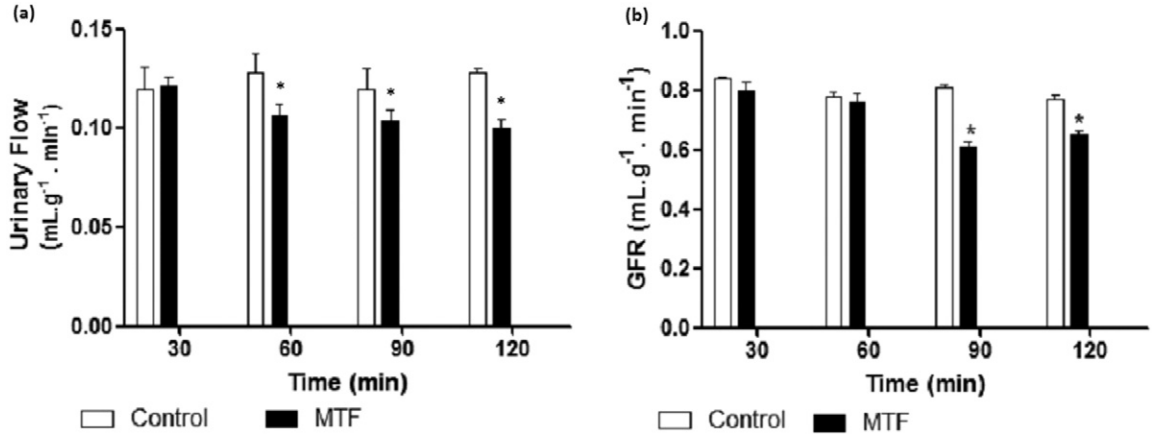

Fig. 1. Determination of urinary flow (a) and glomerular filtration rate — GFR (b) of control and treated with methylphenidate (MPD) groups (N = 6). Results were expressed as mean and standard error of the mean (SEM). *$p < 0.05$ compared with the control group (ANOVA and Bonferroni test).