European Journal of Pharmacology 693 (2012) 72–79

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

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

Endocrine pharmacology

Effect of cimetidine on pentamidine induced hyperglycemia in rats

Toru Arino, Seiji Karakawa, Yasuyoshi Ishiwata, Masashi Nagata, Masato Yasuhara *

Department of Pharmacy, University Hospital of Medicine, Tokyo Medical and Dental University; 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

ARTICLE INFO

Article history:
Received 7 February 2012
Received in revised form
7 July 2012
Accepted 27 July 2012
Available online 21 August 2012

Keywords:
Pentamidine
Cimetidine
Hyperglycemia
Organic cation transporter
Drug interaction
Rat

ABSTRACT

The antiprotozoal agent pentamidine, used for the treatment of *Pneumocystis jirovecii* pneumonia (PCP), is known to cause abnormalities in blood glucose homeostasis, such as hypoglycemia and hyperglycemia. Pentamidine has been reported to be a substrate of organic cation transporter 1 (OCT1). We investigated the combination effects of cimetidine, an OCT1 inhibitor, on the pharmacokinetics of pentamidine and on pentamidine-induced hyperglycemia. Pentamidine was infused intravenously to rats for 20 min at a dose of 7.5 or 15 mg/kg and serum samples were obtained periodically. The serum concentration of glucose did not change significantly after pentamidine infusion at 7.5 mg/kg, while it increased with pentamidine at 15 mg/kg, and the maximal concentration of glucose was 167 ± 36 mg/dl, 30 min after the start of pentamidine infusion. Cimetidine (50 mg/kg) enhanced the pentamidine-induced elevation of glucose concentration and the maximal concentration of glucose was 208 ± 33 mg/dl in the pentamidine 15 mg/kg treated group. Cimetidine combination significantly reduced total body clearance of pentamidine and increased pentamidine concentrations in the liver, kidneys, and lungs. A significant correlation was found between changes in serum glucose concentrations and serum concentrations of pentamidine 30 min after the start of pentamidine infusion. These results suggest that the hyperglycemic effect of pentamidine is dependent on the concentration of pentamidine and can be enhanced by cimetidine combination.

1. Introduction

The antiprotozoal agent pentamidine, an aromatic diamidine, was first used in the 1940s to treat protozoal infections such as trypanosomiasis and leishmaniasis. In the 1950s, pentamidine was reported to have an effect against *Pneumocystis jirovecii* pneumonia (PCP), and has been used for the prevention and treatment of PCP instead of sulfamethoxazole-trimethoprim in patients who have a sulfa allergy or other untoward side effects (Altintas et al., 2011; Crozier, 2011; Kim et al., 2008). In the 1980s, pentamidine treatment for PCP was increased accompanied by an epidemic of acquired immune deficiency syndrome (Conte Jr. et al., 1986, 1987). Recently, biological agents such as antihuman tumor necrosis factor monoclonal antibody, like infliximab or tocilizumab, have become a treatment strategy for rheumatoid arthritis (Ikeuchi et al., 2011; Komano et al., 2009; Wissmann et al., 2011). These agents suppress the immunity of rheumatoid arthritis patients, causing *Pneumocystis jirovecii* infection (Takeuchi et al., 2008), and pentamidine is used for these patients (Komano et al., 2009). Thus, the clinical demand for pentamidine therapy is now increasing.

Pentamidine is known to cause abnormalities in blood glucose homeostasis, such as hypoglycemia and hyperglycemia (Bouchard et al., 1982; Herchline et al., 1991; Lu et al., 1995). The time course of blood glucose concentrations after pentamidine administration tends to show a multiphasic pattern characterized by an early rise lasting for minutes, a subsequent phase of hypoglycemia lasting for hours, and delayed, persistent hyperglycemia. Since the early rise in glucose concentrations was blocked by ergotamine derivatives, it may be partly attributed to an adrenergic reaction (Bouchard et al., 1982; Wien et al., 1943). Serum concentrations of insulin were inappropriately high during the hypoglycemic phase. It has been reported that daily pentamidine administration causes direct β cell toxicity resulting in early insulin release and hypoglycemia, followed by impaired insulin secretion resulting in hyperglycemia (Sands et al., 1985). However, the relationship between pentamidine concentrations and changes in serum glucose concentrations has not been elucidated yet. In the present study, we examined the pharmacokinetics of pentamidine and its effect on serum glucose simultaneously in each individual rat and tried to clarify the pharmacodynamics of pentamidine-induced hyperglycemia.

In vitro studies using transporter-transfected cells have shown that pentamidine is a substrate of organic cation transporter 1 (OCT1) (Jung et al., 2008; Ming et al., 2009). OCTs interact with many cationic drugs, including metformin (Ito et al., 2012;

* Corresponding author. Tel.: +81 3 5803 5601; fax: +81 3 5803 0223.
E-mail address: yasuhara.mpha@tmd.ac.jp (M. Yasuhara).

0014-2999/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2012.07.043

Wang et al., 2002) and oxaliplatin (Yokoo et al., 2007). Oct1 and Oct2 are candidates of basolateral uptake transporters for organic cations in rat hepatocytes (Koepsell et al., 2007). Furthermore, the rat multidrug and toxin extrusion 1 (Mate1) was reported to be the candidate of efflux transporters for organic cations in the liver (Hiasa et al., 2006) and kidneys (Tsuda et al., 2007). Cimetidine is known as an inhibitor of rat Octs (Gründemann et al., 1999) and human OCTs (Iwai et al., 2009; Tsuda et al., 2009). In addition, Ito et al. (2012) recently reported that cimetidine inhibited human MATE1 and mouse Mate1. Therefore, we investigated the combination effects of cimetidine on the pharmacokinetics of pentamidine and on pentamidine-induced hyperglycemia.

2. Materials and methods

2.1. Animals

Male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing 210–260 g were used. Rats were housed in a controlled environment and fasted overnight before the experiment. In in-vivo studies, rats had indwelling cannulas implanted in the left carotid artery and jugular vein under light ether anesthesia for blood sampling and intravenous injection, respectively, and were fasted during the experiment. Animal experiments were performed in accordance with The Guidelines for Animal Experiments of Tokyo Medical and Dental University.

2.2. Materials

Pentamidine isethionate salt was purchased from Sigma-Aldrich Corporation (St. Louis, Missouri, USA). For intravenous infusion, pentamidine isethionate salt was dissolved in saline (0.94 or 1.88 mg/ml). Hexamidine dihydrochloride (as an internal standard for the assay of pentamidine) was purchased from Toronto Research Chemicals Inc. (North York, Canada). Cimetidine chloride was purchased from Nacalai Tesque Inc. (Kyoto, Japan). For intravenous injection, cimetidine chloride was dissolved in saline (5 mg/ml). All chemicals were of analytical grade.

2.3. Intravenous infusion of pentamidine

Pentamidine at 7.5 or 15 mg/kg was intravenously infused for 20 min to rats. Pentamidine doses were determined by reference to a previous study (Assan et al., 1993). An equivalent volume of normal saline was infused to control rats. To determine serum concentrations of glucose, immunoreactive insulin, and pentamidine, blood samples were obtained 0, 10, 19, 23, 30, 50, 80, and 140 min after the start of pentamidine infusion. To determine tissue pentamidine concentrations, samples of tissue (liver, kidneys, and lungs) were obtained 30 or 140 min after the start of pentamidine infusion.

In another set of rats, pentamidine at 7.5 or 15 mg/kg was intravenously infused for 20 min to rats. To determine serum concentrations of histamine, blood samples were obtained 0 and 30 min after the start of pentamidine infusion. An equivalent volume of normal saline was infused to control rats.

2.4. Cimetidine pretreatment

Rats received an intravenous infusion of cimetidine at 50 mg/kg for 3 min. Cimetidine doses were decided according to a previous study (Yanxiao et al., 2011). An equivalent volume of saline was infused to control rats. Five minutes after the end of cimetidine infusion, pentamidine at 7.5 or 15 mg/kg was infused intravenously for 20 min. Blood samples were obtained 0, 10, 19, 23, 30, 50, 80, and 140 min after the start of pentamidine infusion. Samples of tissue (liver, kidneys, and lungs) were obtained 30 or 140 min after the start of pentamidine infusion.

2.5. Analytical methods

Serum concentrations of glucose were determined by the glucose oxidase method using a Glucose CII-test Wako (Wako Pure Chemical Industries, Osaka, Japan). Serum concentrations of immunoreactive insulin were determined by enzyme immunoassay using a Glazyme Insulin-EIA TEST (Wako Pure Chemical Industries).

Serum concentrations of histamine were determined by high performance liquid chromatography (HPLC) with pre-column fluorescence derivatization using the method of Furuhata et al. (1998) with slight modifications. One hundred microliters of serum was mixed with 300 μl of normal saline and 500 μl of 0.05 M phosphate buffer (pH 7.4). The mixture was shaken with 5 ml of 0.05 M bis(2-ethylhexyl)hydrogenphosphate/*n*-heptane for 10 min. After centrifugation at 2000 g for 15 min, the supernatant was acidified with 450 μl of 0.1 M HCl and shaken for 10 min. The aqueous layer, 200 μl, was pipetted out and maintained on ice for 10 min in the presence of 1 M NaOH (40 μl) and 0.1% *o*-phtalaldehyde/methanol (10 μl). After the addition of 3 M HCl (20 μl), 100 μl was injected into the HPLC system. Histamine was detected with a fluorescence detector set at an excitation wavelength of 360 nm and emission wavelength of 450 nm. The mobile phase was acetonitrile, 1 M citric acid 2 sodium-NaOH (pH 5.0), distilled water, and 1-heptanesulfonic acid sodium (180 ml: 10 ml: 810 ml: 1 g). The flow rate was maintained at 1.0 ml/min.

Serum and tissue concentrations of pentamidine were determined by HPLC. For the determination of pentamidine in serum, 150 μl of serum in a 2.0 ml sample tube was added to 15 μl of hexamidine chloride (10 μg/ml), as an internal standard, 30 μl of 1 M sodium hydroxide and 1.2 ml of the extraction solvent consisted of 40% acetonitrile in chloroform. Tubes were vortexed for 30 s, then centrifuged for 5 min at 10,000 g. The upper aqueous layer was discarded, and 1.0 ml of the organic phase was collected into a 1.5 ml sample tube. The contents were taken to dryness at 50 °C. The residue was reconstituted by adding 100 μl of the mobile phase, followed by sonification for 1 min, and 50 μl was injected into the system.

For the determination of pentamidine in the liver, kidneys, and lungs, each tissue was weighed accurately and homogenized with saline (5-fold volume of the each tissue). Then, 150 μl of the homogenate was analyzed in the same way as the preparation of serum samples.

Pentamidine and the internal standard were detected with an ultraviolet detector set at a wavelength of 262 nm. The mobile phase was 20% acetonitrile in 0.1% phosphoric acid and 0.1% sodium chloride. The flow rate was 1.0 ml/min.

The HPLC apparatus was a LC-10A (Shimadzu Co., Kyoto, Japan) equipped with an ultraviolet spectrometer (SPD-6A, Shimadzu Co.) and a spectrofluorometer (RF-550A, Shimadzu Co.). The column was TSK-gel ODS-80T$_M$ (5 μm, 4.6 mm i.d. × 15 cm, TOSOH, Japan) and was kept at 40 °C.

2.6. Pharmacokinetic analysis

The time profiles of serum pentamidine concentrations were fitted to a two-compartment model with intravenous infusion. The pentamidine concentration of a central compartment is described by

$$C_1 = k_0((1-\exp(\alpha T))(k_{21}-\alpha)\exp(-\alpha t)/\alpha(\alpha-\beta) + (1-\exp(\beta T))(k_{21}-\beta)\exp(-\beta t)/\beta(\beta-\alpha))/V_1$$

where k_0 and k_{21} represent the zero-order infusion rate of pentamidine, and the first-order transfer rate constants for a drug