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Differential effects of liver steatosis on pharmacokinetic profile of two closely related hepatoselective NO-donors; V-PYRRO/NO and V-PROLI/NO

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ABSTRACT

Purpose: To analyze the effect of liver steatosis and obesity on pharmacokinetic profile of two structurally-related liver-selective NO-donors – V-PYRRO/NO and V-PROLI/NO.
Methods: C57BL/6 mice were fed control or high-fat diet for 15 weeks to induced liver steatosis and obesity (HFD mice). Pharmacokinetics and renal elimination studies were conducted *in vivo* following *iv* dosing of V-PYRRO/NO and V-PROLI/NO (0.03 mmol/kg). Hepatic clearance was evaluated *ex vivo* in the isolated perfused mice liver and *in vitro* with the use of liver microsomes.
Results: V-PYRRO/NO and V-PROLI/NO, despite similar structure, displayed different pharmacokinetic properties. V-PYRRO/NO was uptaken and metabolized by the liver, while V-PROLI/NO was eliminated unchanged with urine. In HFD mice, despite increased CYP450 metabolism of V-PYRRO/NO the elimination rate was slower most likely due to the impairment of hepatic microcirculation caused by liver fat accumulation. In turn, in HFD mice renal clearence of V-PROLI/NO was accelerated and volume of distribution was increased most likely due to additional intracellular water in HFD mice.
Conclusions: The pharmacokinetics of V-PROLI/NO, the novel proline-based analog of V-PYRRO/NO with additional single carboxylic acid moiety, attached to the molecule of V-PYRRO/NO to improve the water solubility, was differently affected by liver steatosis and obesity as compared with the parent compound V-PYRRO/NO.

Introduction

V-PYRRO/NO (O(2)-vinyl-1-(pyrrolidin-1-yl) diazen-1-ium-1,2-diolate) and V-PROLI/NO (O2-vinyl-[2-(carboxylato)pyrrolidin-1-yl] diazen-1-ium-1,2-diolate) are two structurally similar analogs, members of diazeniumdiolate family, which were designed to release nitric oxide (NO) specifically in the liver, omitting systemic side-effects [1,2]. Compounds were designed to undergo liver biotransformation *via* cytochrome P450 action, by which unstable epoxides are formed, which further spontaneously release NO [1]. It was found that V-PYRRO/NO had protective effects in various *in vitro* and *in vivo* models of hepatotoxicity. For instance, V-PYRRO/NO improved hemodynamics after ischemia reperfusion [3], decreased fibrosis in bile duct ligated rats [4], decreased portal hypertension in cirrhosis [5] and protected liver against acetaminophen induces toxicity [6,7].

V-PROLI/NO is the analog of V-PYRRO/NO with small change in its chemical structure (additional carboxylic moiety), which makes this compound more water soluble. Furthermore, this modification of structure insures better toxicological profile, as nontoxic

Abbreviations: ALT, alanine transaminase; ASP, aspartate transaminase; AUC, area under the concentration-time curve; C_0, initial concentration; Km, Michaelis-Menten constant; LC/MS/MS, liquid chromatography coupled to tandem mass spectrometry; LDH, lactate dehydrogenase; MRT, mean residence time; NAFLDN, onalcoholic Fatty Liver Disease; PBS, phosphate-buffered saline; PK, pharmacokinetics; Vmax, maximal velocity; V-PYRRO/NO, (O(2)-vinyl-1-(pyrrolidin-1-yl) diazen-1-ium-1,2-diolate); V-PROLI/NO, (O2-vinyl-[2-(carboxylato)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate); V_{ss}, volume of distribution in steady-state.

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N-nitrosoproline, instead of N-nitrosopyrrolidine, is formed during V-PROLI/NO metabolism [2]. V-PROLI/NO was considered to be a promising hepatoselective NO-donor, with better *in vitro* NO-releasing properties than V-PYRRO/NO as measured in HepG2 cell line [8] and was showed to protect human liver cells from arsenic toxicity [9]. However, in contrast to V-PYRRO/NO that was shown to be effective against liver steatosis, improving insulin resistance, decreasing fat content in the liver [10], V-PROLI/NO was not effective in this model [11]. These results suggested that only slight difference in the chemical structure of V-PYRRO/NO and V-PROLI/NO results in a considerable difference in their pharmacological activities *in vivo*. Still it is important to note that liver steatosis and obesity may affect the distribution and elimination of V-PYRRO/NO and V-PROLI/NO but this was not as yet characterized.

Pathological changes, associated with obesity, can markedly affect many factors determining pharmacokinetic profile including amount of plasma proteins, drug metabolizing enzymes expression and activity, drug transporters expression and organ blood flow, and therefore may considerably change the distribution, metabolism and elimination of drugs [12]. Taking into consideration, that understanding the differences in pharmacokinetics profile between closely–related structures is of importance for the design of the optimal liver targeted NO donor to treat liver steatosis, the aim of the present work was to evaluate the impact of liver steatosis and obesity on pharmacokinetics and organ elimination of V-PYRRO/NO and V-PROLI/NO, two liver selective NO-donors that differs only by the additional carboxylic acid moiety in V-PROLI/NO.

Materials and methods

Chemicals

Chemicals such as HPLC grade acetonitrile and formic acid were purchased from Merck (Darmstadt, Germany). Sodium chloride, calcium chloride, magnesium sulfate, sodium bicarbonate, potassium dihydrogen phosphate, glucose, pyruvic acid, EDTA, TRIS base, potassium chloride, sucrose, sodium phosphate dibasic, magnesium chloride, NADPH, Folin&Ciocalteu's phenyl reagent, potassium-sodium tartrate tetrahydrate, copper sulfate, sodium hydroxide, 4-hydroxymephenytoin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water used in the study was prepared using a Milli-Q water purification system (Direct-Q 3UV, Millipore, Billerica, MA, USA). Compounds V-PYRRO/NO and V-PROLI/NO (>99% pure) were a kind gift from dr Joseph Saavedra and dr Larry Keefer from Center for Cancer Research NIH (USA).

Animals

Male C57BL/6 mice (Charles River Laboratories, Raleigh, Germany) weighing 16–25 g were divided into two experimental groups and fed the control (AIN-93G) or high fat diet (HFD; 60 kcal % of fat) (Research Diets, USA) for 15 weeks. Prior to experiments, the animals were fasted overnight, but had free access to water. All procedures involving animals were conformed to the Guidelines for Animal Care and Treatment of the European Union and were approved by the Local Ethical Committee for Experiments on Animals.

Pharmacokinetics

The pharmacokinetic profiles of V-PYRRO/NO or V-PROLI/NO were characterized in control or HFD mice after a single intravenous (*iv*) administration of compounds at the dose of 5 mg/kg (0.032 mmol/kg) or 6 mg/kg (0.029 mmol/kg) for V-PYRRO/NO and V-PROLI/NO, respectively. Mice under isoflurane (3–4% in 100% oxygen) anesthesia were sacrificed at the following time intervals: 0 (before dosing) and 2, 5, 7, 10, 15, 20, 25, 30, 45, 60, 90, 120, and 240 min after compounds administration ($n = 4$ mice per time point). Plasma was collected after centrifugation (1000 rpm, 15 min) of blood transferred to heparinized microfuge tubes and frozen at −20 °C prior to analysis.

All data in the pharmacokinetic experiments were processed using Phoenix WinNonlin 6.3 software (Certara, St. Louis, MO, USA). The non-compartmental approach was applied to calculate the basic pharmacokinetic parameters.

Renal clearance

Renal clearances were calculated based on total amount of compounds excreted in urine in unchanged form. Control or HFD mice ($n = 7$) were individually placed in metabolic cages following V-PYRRO/NO (0.032 mmol/kg) or V-PROLI/NO (0.029 mmol/kg) *iv* dosing. The urine samples were collected at 0–6, 6–12, and 12–24 h post dosing. The volumes of the urine samples were recorded, and samples were stored at −20 °C until analysis.

Fraction of administered dose excreted unchanged in urine:

$$f_R = \frac{Ae^{\infty}}{D_{iv}} \tag{1}$$

where f_R is a fraction of dose, which is excreted by kidney, Ae^{∞} is a total mass of drug recovered in urine and D_{iv} is the administered dose.

Renal clearance (Cl_R) of V-PYRRO/NO and V-PROLI/NO was calculated based on the following equations:

$$Cl_R = f_R \times Cl_T \tag{2}$$

$$Cl_R = \frac{Ae^{\infty}}{AUC_0^{\infty}} \tag{3}$$

Hepatic clearance

Hepatic extraction ratio and hepatic clearance were determined using the *ex vivo* isolated perfused mouse liver model. Mice were anesthetized with an *ip* injection of ketamine (100 mg/kg), xylazine (10 mg/kg). Following *ip* dosing of heparin (0.8 mg/kg), the *vena portae* and the *vena cava inferior* were cannulated and ligated, and the livers were perfused with Krebs-Hanseleit buffer to remove all blood (Hugo Sachs Elektronik, Harvard Apparatus, March-Hugstetten, Germany). Afterwords the livers were excised and moved to a moist chamber. Following further initial stabilization period (15 min), either V-PYRRO/NO or V-PROLI/NO was added at a final concentration of 10 μM and the perfusions were carried out for 1 h in a single pass set up ($n = 4$ mice per compound). The perfusion flow rate was set to 4 mL/min/g of tissue, and samples were collected as follows: inlet samples every 20 min, and outlet effluents every 1 min, between 5 and 20 min, and then every 10 min. After the experiments, the livers were excised, dried, and weighed.

Hepatic extraction ratio for V-PYRRO/NO and V-PROLI/NO were calculated on the basis of the difference in their concentrations in inlet and outlet effluents from the liver:

$$E = \frac{C_{in} - C_{out}}{C_{in}} \tag{4}$$

where E is the hepatic extraction ratio and C_{in} and C_{out} are concentrations of studied compounds in inlet or outlet liver effluents.