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Glucosamine modulates propranolol pharmacokinetics *via* intestinal permeability in rats

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ABSTRACT

Propranolol (PROP) undergoes extensive first-pass metabolism by the liver resulting in a relatively low bioavailability (13–23%); thus, multiple oral doses are required to achieve therapeutic effect. Since some studies have reported that glucosamine (GlcN) can increase the bioavailability of some drugs, therefore, it is aimed to study whether GlcN can change the pharmacokinetic parameters of PROP, thus modulating its bioavailability. When PROP was orally co-administered with GlcN (200 mg/kg) to rats, PROP area under curve (AUC) and maximum concentration (C_{max}) were significantly decreased by 43% ($p < 0.01$) and 33% ($p < 0.05$), respectively. In line with the *in vivo* results, *in silico* simulations confirmed that GlcN decreased rat intestinal effective permeability (P_{eff}) and increased PROP clearance by 50%. However, *in situ* single pass intestinal perfusion (SPIP) experiments showed that GlcN significantly increased PROP serum levels ($p < 0.05$). Furthermore, GlcN decreased PROP disposition/distribution into cultured hepatocytes in concentration dependent manner. Such change in the interaction pattern between GlcN and PROP might be attributed to the environment of the physiological buffer used in the *in vitro* experiments (pH 7.2) *versus* the oral administration and thus, enhanced PROP permeability. Nevertheless, such enhancement was not detected when everted gut sacks were incubated with both drugs at the same pH *in vitro*. In conclusion, GlcN decreased PROP serum levels in rats in a dose-dependent manner. Such interaction might be attributed to decreased intestinal permeability and enhanced clearance of PROP in the presence of GlcN. Further investigations are still warranted to explain the *in vitro* inhibitory action of GlcN on PROP hepatocytes disposition and the involvement of GlcN in the intestinal and hepatic metabolizing enzymes of PROP at different experimental conditions.

1. Introduction

Beta-blockers have been widely used for the treatment of many cardiovascular diseases (Mansoor and Kaul, 2009; Reiter, 2004). In humans, PROP is the first non-selective β-adrenergic blocker used for the treatment of essential hypertension, arrhythmias, congestive heart failure, and myocardial infraction (Chafin et al., 1999; Stephenson et al., 1980; Wang et al., 2013). Moreover, it has been proved that PROP possesses anti-inflammatory, antioxidant properties, lipid peroxidation inhibitory effect as well as anti-cancer activities (Nkontchou et al., 2012). PROP is also effective clinically in the treatment of some neurologic diseases, such as headache and migraine (Katzung et al., 2004; Shields and Goadsby, 2005).

PROP is a highly lipophilic drug that is almost completely absorbed from the gastrointestinal tract following oral administration (Salman et al., 2010). According to the Biopharmaceutics Classification System (BCS), PROP is classified as class 1 drug with rapid dissolution, high-solubility and high-permeability (Custodio et al., 2008). However, PROP in man is highly metabolized by the liver as it undergoes extensive first-pass metabolism resulting in a relatively low oral bioavailability of 13–23% (Cid et al., 1986; Ismail et al., 2004; Ludden, 1991; Sastry et al., 1993) with a half-life ranging from 3 to

Abbreviations: PROP, propranolol; GlcN, glucosamine; CIME, cimetidine; RIFA, Rifampin; SPIP, single-pass intestinal perfusion; ERIS, everted rat intestinal sac; SLS, sodium lauryl sulphate; HPV, hepatic portal vein; IVC, inferior vena cava; NCA, non-compartmental analysis; C_{max}, maximum concentration; T_{max}, time of higher concentration; AUC, area under the curve; AUMC, area under the first moment curve; MRT, mean residence time; Kel, the fraction of drug eliminated per time; $t_{0.5}$, elimination half-life; P_{eff}, rat intestinal effective permeability; V_d, volume of distribution; h, hour; min, minute; BCS, biopharmaceutics classification system

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6 h (Castleden and George, 1979; Ismail et al., 2004; Leahey et al., 1980) and a large volume of distribution (V_d) of 4 L/kg (Ismail et al., 2004). Thus, it shows marked variations in its bioavailability among patients. As a result, the connection between the amount of dose and the bioavailability of PROP reached after oral administration is still ambiguous (Kiriyama et al., 2008). Few studies have been conducted on rats to increase PROP bioavailability. For instance, Ryu et al. used rectal route of PROP and found the latter route yielded higher bioavailability of PROP than oral administration which may reflect partial bypassing hepatic first pass metabolism (Ryu et al., 1999; van Hoogdalem et al., 1991). Another study in dogs has shown that co-administering PROP with a lipid vehicle increased its bioavailability mainly by increasing PROP intestinal absorption (Aungst and Hussain, 1992). Therefore, PROP bioavailability can be increased in rats by either partial bypassing hepatic first pass metabolism and/or increasing PROP intestinal absorption.

Glucosamine, 2-amino-2-deoxy-D-glucose, (GlcN) is a monosaccharide compound generated by hydrolysis of chitosan or chitin and is considered as an important amino sugar building block for mucoproteins, mucolipids and mucopolysaccharides (Al-Hamidi et al., 2010; Kirkham and Samarasinghe, 2009; Xing et al., 2006). GlcN is produced naturally in the cells as GlcN-6-phosphate *via* hexosamine biosynthetic pathway and is involved in the formation of glycolipids, glycosaminoglycans, and proteoglycans. This sugar is further used for O-linked glycosylation of several proteins causing changes in the biological activity (Anderson et al., 2005; Roseman, 2001; Uldry et al., 2002). Furthermore, several studies have reported the involvement of GlcN in modulating drug pharmacokinetics and pharmacodynamics. For example, GlcN combination with other non-opioid analgesics was found to have synergistic, sub-additive, or additive effect as well as pain relieving effect depending on the ratios of GlcN and certain non-steroidal anti-inflammatory drugs (NSAIDs) (Tallarida et al., 2003). Yet, most of the previously reported investigations were based on pharmacodynamics actions. It has been previously reported that GlcN, to some extent, is capable of competing with the paracetamol molecules on the catalytic pocket of the human CYP2E1 protein and concluded that GlcN increased paracetamol bioavailability by decreasing its metabolism (Qinna et al., 2015). The same study confirmed that GlcN also reduced hepatocyte injury after the administration of high doses of paracetamol (Qinna et al., 2015). Therefore, the aim of this study was to evaluate the effect of GlcN on PROP absorption in rats by investigating whether there is any possible pharmacokinetic interaction between PROP and GlcN *in vivo*, *in situ* and *in vitro*. PROP-GlcN pharmacokinetic interaction was compared with the other known interactors; rifampicin (RIFA), a hepatic enzyme inducer that has been demonstrated to accelerates the metabolism of several drugs (Brunton et al., 2006), and cimetidine (CIME), a hepatic enzyme inhibitor responsible for PROP metabolism (Reimann et al., 1981).

2. Materials and methods

2.1. Materials

Potassium chloride and ethylene diamine tetra acetic acid were purchased from Acros organics (BVBA Geel, Belgium). Nanopur™ deionized water, methanol advanced gradient grade, and acetonitrile were obtained from Fisher Scientific Ltd. (Loughborough, UK). Potassium dihydrophosphate, penicillin, streptomycin, fetal bovine serum, sodium lauryl sulphate, and magnesium sulphate were all purchased from Sigma-Aldrich (St. Louis, Missouri), sodium bicarbonate from Merck (Darmstadt, Germany), phosphoric acid from Kyowa Medex Co. (Tokyo, Japan), and triethylamine from Tedia Company, Inc. (USA) were also used. All chemicals were of analytical grade, whereas solvents were of HPLC grade. For hepatocyte isolation and incubation, Hank's balanced salt solution (HBSS), without and with Ca^{2+} and Mg^{2+}, and Williams's medium E were obtained from Invitrogen (Carlsbad, CA, USA), whereas collegenase II and L-glutamine were purchased from Gibco BRL (Gaithersburg, MD, USA).

PROP-HCl was a kind gift from The Arab Pharmaceutical Manufacturing (APM) (Salt, Jordan), whereas GlcN-HCl was obtained from Biocon (Bangalore, India; batch No: DA-B10-04-000650/02493). Sildenafil citrate and Tris base were kindly obtained from the Jordanian Pharmaceuticals Manufacturing company (JPM) (Amman, Jordan). RIFA and Florane® (isoflurane) were a kind gift from Hikma Pharmaceuticals (Amman, Jordan), whereas CIME was kindly donated by Jordan Sweden Medical and Sterilization Company (JOSWE) (Naur, Jordan).

2.2. Animals

The protocols for the animal study were approved by the Ethics Committee of the Research Council at the Faculty of Pharmacy and Medical Sciences, University of Petra (Amman, Jordan). Adult male and non-pregnant female Sprague Dawley rats were supplied and housed at the Animal House of University of Petra. Rats with average weight of (220 ± 20 g) were used. Rats were kept in air-conditioned environment under controlled temperatures (22–24C), humidity (55–65%), and photoperiod cycles (12 h light/12 h dark). Rats were kept fasting overnight (for 18 to 22 h) without free access to water, unless otherwise stated. All experiments were achieved in accordance with the Institutional Guidelines on Animal Use of University of Petra, which adopts the guidelines of the Federation of European Laboratory Animal Science Association (FELASA).

2.3. In vivo GlcN, CIME and RIFA effect on PROP serum levels in rats

Reference solutions of PROP, CIME, RIFA and GlcN were prepared by dissolving an accurately weighed amount of each in distilled water to obtain 4, 1, 2 and 40 mg/ml, respectively. All solutions were freshly prepared on the day of experiment and were administered to fasting rats by stainless steel oral gavage needles (Harvard Apparatus, Kent, UK). For all experiments, rats were marked on tail for identification, weighed, and randomized into four groups consisting 7 rats/group. Before administration, blood samples were pooled from lateral tail vein of all grouped rats. Later, the rats received 1 ml of either water alone, GlcN 40 mg/ml, CIME 1 mg/ml or RIFA 2 mg/ml. After 30 min, all groups received 4 mg/ml of PROP solution. Rats in GlcN group were maintained on drinking water containing 25 g/l GlcN for three days prior to GlcN administration. Rats in RIFA experiment received a daily single dose of RIFA solution 2 mg/ml for two weeks before the day of experiment (Herman et al., 1983). For all groups, blood samples collected at different time intervals (0.25, 0.5, 1, 2, 3, 6, 8 and 10 h). Blood was left to clot, centrifuged for 10 min at 14000 rpm. Then, serum was separated, transferred directly into eppendorf tubes, and kept in freezer at − 20 °C until HPLC analysis. Six independent experiments were performed and analyzed for each group (n = 6).

2.4. HPLC analysis

HPLC system used from Thermo Separation Products (Waltham, MA, USA) was set at a wavelength of 214 nm and coupled to a BDS hypersil C18 column (150 × 4.6 mm^2 and particle size 5 µm) from Thermo Electron Corporation (San Jose, CA, USA) with a flow rate of 1 ml/min. The mobile phase was prepared as a mixture of acetonitrile, methanol and triethylamine phosphate solution (15: 32.5: 52.5, v/v). Triethylamine phosphate solution was prepared by the addition of 900 µl triethylamine to 1 l of water then pH was adjusted to 2.75 using phosphoric acid. A volume of 100 µl of serum or Krebs buffer samples (pH 7.2) were transferred to tubes followed by the addition of 150 µl of the internal standard sildenafil (5.0 µg/ml) dissolved in acetonitrile. Samples were vortexed for 1 min and centrifuged at 14000 rpm for 15 min. The supernatant was injected to HPLC using 25 µl injection