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Research paper

Mild hypothermia decreases the total clearance of glibenclamide after low dose administration in rats

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HIGHLIGHTS

- Compared with normothermia, mild hypothermia significantly decreased the total clearance of glibenclamide.
- Mild hypothermia reduced the total clearance of diclofenac, indicating that the CYP2C9 activity was compromised in reduced temperature.
- Mild hypothermia alters the pharmacokinetics of glibenclamide, probably via mediating the activity of CYP2C9.

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ABSTRACT

Background and purpose: Low dose glibenclamide exhibits pleiotropic protective effects in different central nervous system diseases. Previously, we have shown that mild hypothermia enhanced the efficacy of glibenclamide in the cultured cortical neuronal cells. This study aims to evaluate the impact of mild hypothermia on the pharmacokinetics of low dose glibenclamide in rats via its cytochrome P450 2C9 (CYP2C9) metabolic pathway.

Methods: Male Sprague-Dawley rats were maintained at 37 °C (normothermic group) or cooled to 33 °C (hypothermic group). Glibenclamide (33 μg/kg) or diclofenac (10 mg/kg, a probe drug for assessing the activity of CYP2C9 which involves in glibenclamide and diclofenac metabolism in liver) were intravenously administered at 10 min after stabilization of temperature. Plasma samples were collected at 9 different time points. Glibenclamide and diclofenac in sera were separated by liquid chromatography and quantified with tandem mass spectrometry.

Results: Compared with normothermia, mild hypothermia significantly decreased the total clearance of glibenclamide (16.00 ± 4.1–6.72 ± 2.1 mL/min/kg; $p < 0.01$), and there was a non-significant trend in a slightly higher half-life, (1.64 ± 0.34–2.71 ± 1.7 h, $p = 0.157$). Area under the plasma concentration versus time curve (AUC_{last}) in the hypothermic group was increased (33.2 ± 11–77.8 ± 18 h ng/mL, $p < 0.01$). Moreover, mild hypothermia reduced the total clearance of diclofenac (10.33 ± 1.53–7.20 ± 1.66 mL/min/kg, $p < 0.01$), indicating that the CYP2C9 activity was compromised in reduced temperature.

Conclusion: Mild hypothermia reduced the total clearance of glibenclamide, probably via mediating the activity of CYP2C9. The impact of hypothermia in clinical application of glibenclamide should be considered.

1. Introduction

Best known as a hypoglycemic agent since 1969, glibenclamide (US adopted name, glyburide) has contributed prominent to the treatment of diabetes mellitus type II, via promoting insulin release by blocking pancreatic sulfonylurea receptor 1 (SUR1)-Kir6.2 (K_{ATP}) channel [1]. However, lethal hypoglycemia in some cases with the treatment dose of glibenclamide has limited its clinical application [2]. In recent years, interest in this agent has been rekindled due to the administration of low dose glibenclamide providing neuroprotection in different central nervous system (CNS) pathologies such as ischemic stroke, traumatic brain injury and spinal cord injury, via inhibition of the SUR1-transient receptor potential M4 (TRPM4) channel [3–7]. Moreover, retrospective studies on stroke patients with diabetics as well as prospective clinical trial in malignant

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edema and stroke indicate that glibenclamide is a highly promising drug for the treatment of stroke [8,9].

Mild hypothermia, usually defined as controlled body temperature down to 32–34 °C, is recommended as a clinical intervention for hypoxic-ischemic encephalopathy [10]. Experimental data in animal studies demonstrated that hypothermia is a promising therapeutic strategy in acute ischemic stroke [11–13]. A clinical trial on 1400 patients (European multicenter clinical trial) to evaluate this is ongoing to clarify the validity of hypothermia [14]. Both glibenclamide and hypothermia are experimental in stroke patients.

Mild hypothermia alters the pharmacokinetics of many medications commonly used in patients with CNS injury [15,16]. For example, mild hypothermia increases dopamine half-life in pigs, extends duration of atracurium action and reduced rate of effect site equilibration and clearance of vecuronium in healthy volunteers [17–19]. Therapeutic hypothermia also significantly reduces phenytoin elimination in children with severe traumatic brain injury [20]. Our previous study suggests that combined treatment with glibenclamide and mild hypothermia promotes cultured neuronal cell survival under oxygen–glucose deprivation and reoxygenation stress [21]. However, whether the metabolism of glibenclamide was affected by mild hypothermia is still unknown. A comprehensive understanding of the pharmacokinetics of glibenclamide in mild hypothermia might ensure the combined treatment of low dose glibenclamide and mild hypothermia both in the continued in vivo study and their future clinical application.

Cytochrome P450 (CYP) 2C9 is an important member in CYP family that primarily expressed in the liver, and the expression level is reported to be the second highest among CYP isoforms [22]. It plays a major role in the metabolism of multiple compounds such as phenytoin, S-warfarin, celecoxib, ibuprofen, diclofenac, as well as glibenclamide [23]. CYP2C9 is mainly responsible for the hepatic metabolism of glibenclamide [24]. Therefore, we logically proposed that mild hypothermia might alter the pharmacokinetics of glibenclamide via mediating the activity of CYP2C9. To verify this hypothesis, we used sensitive liquid chromatography tandem triple quadrupole mass spectrometry (LC–MS/MS) to evaluate the pharmacokinetics of low dose glibenclamide under different temperature conditions in rats, and we assessed the activity of CYP2C9 under mild hypothermia by measuring the clearance of diclofenac, a well-known probe drug to reflect the CYP2C9 enzymatic activity [25].

2. Materials and methods

2.1. Hypothermia protocol

Animal experiments were performed under a protocol approved by the Institutional Animal Care and Use Committee of Southern Medical University. Twenty-four healthy adult male Sprague-Dawley rats (200–250 g) were acclimated for 5 days prior to study initiation. The animals were housed on a 12 h light/dark cycle. Rats were randomized to mild hypothermia (33 ± 0.5 °C) or normothermia (37 ± 0.5 °C) with target temperatures. After anesthetization, rats in the hypothermia group were immediately cooled to 33 ± 0.5 °C with ice bags, then the temperature were maintained with a heating pad regulated by rectal temperature. For rats in the normothermia group, rectal temperature was maintained at 37 ± 0.5 °C with a heating lamp. During the entire period of drug administration and blood collection, rats remained hypothermic or normothermia as appropriate.

2.2. Drug administration and pharmacokinetic sampling

Twenty-four rats were randomized to 4 groups, in a 2 × 2 matrix, receiving treatment of: mild hypothermia or normothermia and either glibenclamide (Sigma–Aldrich, St. Louis, MO, USA) or diclofenac (Sigma–Aldrich) respectively. The sample size was according to the Drug Non-clinical Pharmacokinetic Research Technical Guidelines (The 2014 edition) of China Food and Drug Administration (CFDA) for drug non-clinical pharmacokinetic researches. At 10 min after stabilization of rectal temperature, 33 μg/kg glibenclamide or 10 mg/kg diclofenac were intravenous injected respectively [5]. Nine time points to collect the blood samples (0.3 mL) included 5 min, 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 5 h and 8 h. Equal volume of saline was supplemented to animals after each collection. After centrifugation, the clear supernatant was stored at −80 °C. Blood glucose was monitored at each time point.

2.3. Drug analysis by LC–MS/MS

Glibenclamide concentration in rat plasma was analyzed by LC–MS/MS using modifications of a previous established method [26]. The analyte was extracted by protein precipitation, using propranolol (Sigma–Aldrich) as internal standard. The LC system was coupled to an AB Sciex QTrap 4000 mass spectrometer equipped with an electrospray ionization source (Framingham, MA, USA). Following the sample preparation, 2 μL of each sample was injected onto an Xbridge-C18 3.5 μm, 2.1 × 50 mm column (Waters, Milford, MA, USA). Mobile phase A consists of 2 mM ammonium formate/ultra purified water and 0.1% formic acid, while mobile phase B consists of 2 mM ammonium formate/methanol and 0.1% formic acid. Each injection had 5 min run time. Positive ionization was selected for MS detection. A multiple reaction monitoring (MRM) function was used for quantification, with the iron transition set at m/z 494 → 369.

The liquid–liquid extraction method was employed in the preparation of diclofenac plasma samples. D5-t1116 (an undocumented agent) was selected as internal standard. The mobile phase A was 0.1% ammonia/ultra purified water, and the mobile phase B was 0.1% ammonia/methanol. The analyte was tested using negative ionization mode. Iron transition was set at m/z 295 → 251.

The mass spectrometric conditions were optimized for both glibenclamide and diclofenac, including declustering potential (100, −52 V), collision energy (19, −15 V), ion spray voltage(5500, −4500 V), entrance potential (10, −10 V), and collision exit potential (12, −12 V) respectively. Nitrogen was employed as the nebulizing gas.

Calibration curves for glibenclamide and diclofenac were linear from 0.5–50 ng/mL (R^2 = 0.9973) and 10–40,000 ng/mL (R^2 = 0.9961) with lower limits of quantification of 0.5 ng/mL and 10 ng/mL, respectively. Inter- assay and intra-assay variation was <15%.

2.4. Pharmacokinetic analysis

The pharmacokinetic parameters were estimated by non-compartmental methods using WinNonlin software (Pharsight Corporation, Mountain View, CA, USA), including elimination half-life time ($t_{1/2}$), area under the plasma concentration versus time curve from zero to last (AUC_{last}) and total clearance. Plasma concentration versus time profiles were analyzed simultaneously.

2.5. Statistical analysis

All data were presented as means ± standard deviations and analyzed with unpaired Student's t-tests, using SPSS 20.0 (IBM, Armonk, NY). A p-value less than 0.05 was considered statistically significant.