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Model based population PK-PD analysis of furosemide for BP lowering effect: A comparative study in primary and secondary hypertension

Mahendra Shukla[a,b], Moustafa M.A. Ibrahim[c,d], Moon Jain[b,e], Swati Jaiswal[a,b], Abhisheak Sharma[a,b,f], Kashif Hanif[b,e], Jawahar Lal[a,b,*]

[a] Pharmacokinetics & Metabolism Division, CSIR-Central Drug Research Institute, Lucknow 226031, India
[b] Academy of Scientific and Innovative Research, Mathura Road, New Delhi 110025, India
[c] Department of Pharmaceutical Biosciences, Uppsala University, Uppsala 75124, Sweden
[d] Department of Pharmacy Practice, Helwan University, Cairo-11175, Egypt
[e] Pharmacology Division, CSIR-Central Drug Research Institute, Lucknow 226031, India
[f] Department of Pharmaceutics, University of Florida, Gainesville, FL -32610, USA[1]

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ABSTRACT

Though numerous reports have demonstrated multiple mechanisms by which furosemide can exert its anti-hypertensive response. However, lack of studies describing PK-PD relationship for furosemide featuring its anti-hypertensive property has limited its usage as a blood pressure (BP) lowering agent. Serum concentrations and mean arterial BP were monitored following 40 and 80 mg kg^{-1} multiple oral dose of furosemide in spontaneously hypertensive rats (SHR) and DOCA-salt induced hypertensive (DOCA-salt) rats. A simultaneous population PK-PD relationship using E_{max} model with effect compartment was developed to compare the anti-hypertensive efficacy of furosemide in these rat models. A two-compartment PK model with Weibull-type absorption and first-order elimination best described the serum concentration-time profile of furosemide. In the present study, post dose serum concentrations of furosemide were found to be lower than the EC_{50}. The EC_{50} predicted in DOCA-salt rats was found to be lower (4.5-fold), whereas the tolerance development was higher than that in SHR model. The PK-PD parameter estimates, particularly lower values of EC_{50}, K_e and Q in DOCA-salt rats as compared to SHR, pinpointed the higher BP lowering efficacy of furosemide in volume overload induced hypertensive conditions. Insignificantly altered serum creatinine and electrolyte levels indicated a favorable side effect profile of furosemide. In conclusion, the final PK-PD model described the data well and provides detailed insights into the use of furosemide as an anti-hypertensive agent.

1. Introduction

Diuretics with a clinical history of several decades serve as an important/integral and multifaceted class of anti-hypertensive drugs. Several studies have shown that early start of diuretic therapy is highly beneficial in a large subgroup of hypertensive populations. Amongst the members of this class, thiazide diuretics are the most widely prescribed; however, several side effects (kidney damage, hyperglycemia, hyperuricemia, hypokalemia, *etc.*) limit their clinical applicability (Ellison and Loffing, 2009; Huen and Goldfarb, 2007). As a better alternative to thiazides, loop diuretics have potential to be included in anti-hypertensive regimen because of their blood pressure (BP) lowering property along with a preferable side effect profile.

It has been shown that the BP lowering effect of loop diuretics is not solely dependent on their diuretic properties. The off-target class effects including vasodilation, modulation of renin expression, renal perfusion

Abbreviations: (SHR), Spontaneously hypertensive rats; (DOCA-salt rats), Deoxycorticosterone acetate-salt induced hypertensive rats; (PK-PD), Pharmacokinetic-pharmacodynamic; (NONMEM), Nonlinear mixed effect modeling; (BP), Blood pressure; (ESH/ESC), European society of hypertension/cardiology; (JNC), Joint national committee; (MAP), Mean arterial blood pressure; (LC-MS/MS), Liquid chromatography-tandem mass spectrometry; (NIBP), Noninvasive blood pressure monitoring; (linear ion trap), LIT; (ESI), Electrospray ionization; (MRM), Multiple reaction monitoring; (CAD), Collision activated dissociation; (LLE), Liquid-liquid extraction; (OFV), Objective function value; (VPC), Visual predictive checks; (TAD), Time after dose; (CL), Clearance; (V1 and V2), Volume of distribution of central and peripheral compartment; (Q), Inter-compartmental clearance; (ω), Between-subject variability; (σ), Residual variability; (K_e), Rate constant for drug transfer from central to effect compartment; (EC_{50}), Concentration at half maximal effect; (TOLE), Tolerance; (C_e), Effect compartment drug concentration

* Corresponding author at: Pharmacokinetics & Metabolism Division, CSIR-Central Drug Research Institute, Jankipuram Extension, Sitapur Road, Lucknow 226031, India.
E-mail address: j_lal@cdri.res.in (J. Lal).
[1] Present Affiliation

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Table 1
Dosing schedule of furosemide in rats for PK-PD study.

Group (n = 8)	Rat model	Dosing schedule
Pharmacokinetic study		
I	Wistar rats (normotensive control)	40 mg kg^{-1} day^{-1}, per oral, for 2 weeks
II	Wistar rats (normotensive control)	80 mg kg^{-1} day^{-1}, per oral, for 2 weeks
III	SHR	40 mg kg^{-1} day^{-1}, per oral, for 3 weeks
IV	SHR	80 mg kg^{-1} day^{-1}, per oral, for 3 weeks
V	DOCA-salt rats	40 mg kg^{-1} day^{-1}, per oral, for 2 weeks
VI	DOCA-salt rats	80 mg kg^{-1} day^{-1}, per oral, for 2 weeks
Pharmacodynamic study		
VII	SHR (control)	Saline, per oral, for 3 weeks
VIII	SHR	40 mg kg^{-1} day^{-1}, per oral, for 3 weeks
IX	SHR	80 mg kg^{-1} day^{-1}, per oral, for 3 weeks
X	DOCA-salt rats (control)	Saline, per oral, for 2 weeks
XI	DOCA-salt rats	40 mg kg^{-1} day^{-1}, per oral, for 2 weeks
XII	DOCA-salt rats	80 mg kg^{-1} day^{-1}, per oral, for 2 weeks

and glomerular filtration, *etc.* have also been recognized for their anti-hypertensive action (Hocherl et al., 2004; Orlov et al., 2010; Tamargo et al., 2014). The European society of hypertension/cardiology (ESH/ESC) 2007 guidelines had listed loop diuretics as preferred diuretics for hypertensive patients with end-stage renal disease, proteinurea or heart failure (Mancia et al., 2007; Mancia et al., 2013). However, due to the paucity of outcome data, joint national committee (JNC) 8 has not prioritized loop diuretics in any subgroup of hypertensive patients (Chobanian et al., 2003; James et al., 2014).

Furosemide, a potent loop diuretic, acts by inhibiting $Na^+ - K^+ - 2Cl^-$ symport. Apart from its usage to treat fluid retention in patients with chronic kidney disorders and congestive heart failure, it has also been used as an adjuvant therapy to treat hypertension (Terland, 1990). Due to its natriuretic and blood volume reducing effect, it has also been used in patients with resistant hypertension. In fact, in a clinical study by Davidov et al. (1967) involving 113 hypertensive patients, furosemide at high doses was found to affect mean arterial pressure greater than any dose of the thiazides irrespective of its diuretic effect. Though, furosemide increased the urine volume and decreased the plasma volume but, no direct relation was observed between these parameters and fall in arterial BP.

Despite its long term use as an established diuretic, the anti-hypertensive effect of furosemide has not been well characterized. The literature survey revealed that the previous pharmacokinetic-pharmacodynamic (PK-PD) studies of furosemide mainly modeled its diuretic effect (Hammarlund et al., 1985; Jang et al., 1994). The reports pertaining to the use of furosemide in treating different forms of hypertension, broadly primary (environmental or genetic origin) and secondary hypertension (due to identifiable cause like renal, vascular and endocrine dysfunctions, *etc.*) are limited. Also, the multiple dose studies for the anti-hypertensive effect of furosemide are lacking. Therefore, the present study aims at bridging this void by providing a comparative PK-PD analysis for the BP lowering effect of furosemide after multiple dosing in spontaneously hypertensive rats (SHR) and deoxycorticosterone acetate-salt-induced hypertensive rats (DOCA-salt rats), the commonly employed animal models for human primary and secondary hypertension, respectively (Doggrell and Brown, 1998; Pinto et al., 1998; Yang et al., 2014). It will provide deep understanding of the mechanism behind anti-hypertensive action of furosemide to define the adequate dosage regimen.

2. Material and Methods

2.1. Materials

Furosemide (purity ≥ 98.0%) was obtained as gratis sample from Alpa Laboratories Limited, India. Curcumin (purity ≥ 98.0%), DOCA and sodium chloride (NaCl) were purchased from Sigma Aldrich (St. Louis, USA). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) grade acetonitrile (ACN) and analytical grade ammonium formate were purchased from Sigma Aldrich (Domdivli, India). HPLC grade ethyl acetate was procured from Spectrochem (Mumbai, India). Ultrapure water (18.2 ΩM) was from a Milli-Q PLUS PF (Billerica, USA) water purification system.

Drug free blood was collected from young and healthy male SHR and Wistar rats provided by the Laboratory Animal Services Division of the institute and serum was separated after centrifuging the collected blood at 3000*g* for 10 min. Following selectivity screening, the collected serum was pooled and stored at − 80 °C till use. SHR with mean arterial BP (MAP) of > 160 mmHg were used in the study. Hypertension (MAP > 160 mmHg) was developed in Wistar rats by twice a week subcutaneous injections of DOCA (25 mg kg^{-1}) and salt loading as 1% NaCl in the drinking water for two months (Doggrell and Brown, 1998). These DOCA-salt rats with MAP > 160 mmHg were used for the study. All experiments, euthanasia and disposal of carcasses were carried out as per the guidelines of the local ethics committee for animal experimentation.

2.2. Experimental Design

A multiple oral dose PK-PD study of furosemide was performed in male SHR, DOCA-salt and Wistar (normotensive control) rats. As suggested in the literature (Hammarlund and Paalzow, 1982; Jang et al., 1994), the diuretic effect of furosemide has been previously explored at 40 mg kg^{-1} dose and for the purpose of exploring its anti-hypertensive effect at a higher dose; we conducted our studies at an additional 80 mg kg^{-1} dose as well. The rats were divided into 12 groups (each n = 8) as described in Table 1. Groups I - VI rats were used for PK study whereas, groups VII–XII rats were used for PD study. For PK study, the overnight-fasted (14–16 h; free access to water) rats received the suspension formulation of furosemide as scheduled in Table 1. The suspension formulation for oral dosing of furosemide was prepared in Milli-Q water using gum acacia (1% w/v). Blood (~100 µL) was collected in microtubes (Axygen, California, USA) from the caudal vein by excising the tail at 0.5, 1, 2, 4, 8, 24, 27, 74, 146, 218, 314, 410, 532, 546 and 552 h post dose. The blood was allowed to clot, centrifuged at 3000*g* for 10 min at 4 °C and the serum was separated and stored at − 80 °C until analysis. Test samples (10 µL) were assayed along with calibration standards and QC samples prepared in rat serum using developed and validated LC-MS/MS method and the levels of furosemide were calculated using Analyst™ (version 1.4.2; Applied Biosystems, Toronto, Canada). The MAP of groups VII–XII rats was monitored using noninvasive BP monitoring (NIBP; Columbus Instruments, OH, USA) following oral administration of furosemide suspension as per the dosing schedule mentioned in Table 1. NIBP was monitored in conscious rats using tail cuff method at various predefined time points till a