Research article

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DEPRESSION-LIKE EFFECT OF LOSARTAN POTASSIUM ON IMMOBILITY IN MICE: PRELIMINARY EVIDENCE FOR THE INVOLVEMENT OF ITS ACTIVE METABOLITE EXP 3174

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ABSTRACT

The effect of acute oral treatment of losartan potassium, an angiotensin AT_1 receptor blocker, on immobility in the forced swim test have been studied using rifampicin-treated (cytochrome P-450 enzyme-induced) and fluconazole-treated (cytochrome P-450 enzyme-inhibited) swiss albino mice respectively. In vehicle-treated group, an enhancement in immobility time was observed at 3 h and 6 h after losartan potassium treatment (20 mg/kg, p.o) in the mouse forced swim test. In rifampicin-treated group, even at 1 h after losartan potassium treatment (20 mg/kg, p.o.) a significant enhancement in immobility was observed. It has also been observed that the basal immobility time of rifampicin-treated mice was significantly ($p<0.001$) lower when compared with vehicle treated group. In fluconazole-treated group, losartan potassium (20 mg/kg, p.o.) could not significantly alter the immobility time at any point of time. The present study results suggest that the depression-like effect of losartan potassium might be mainly mediated by its active metabolite EXP 3174. However, further studies using EXP 3174 are warranted to confirm its CNS activities.

Keywords: immobility time, losartan potassium, EXP 3174, rifampicin, fluconazole, cytochrome P-450 enzyme

INTRODUCTION

The renin angiotensin system (RAS) is commonly known for its action to regulate the salt and water homeostasis. Angiotensin II (Ang II) is an effector peptide of the RAS, acts on both AT_1 and AT_2 receptors which are coupled with G protein. Activation of AT_1 receptor mediates the classical peripheral effects such as a renal salt and water retention, vasoconstriction, and facilitation of sympathetic transmission [1]. Interestingly, a separate RAS within the mammalian brain was identified and demonstrated the presence of precursors and enzymes responsible for the formation and deactivation of angiotensin in the brain [1, 2, 3].

In our previous study, losartan potassium at high doses (20 and 100 mg/kg, i.p) significantly enhanced the immobility time even after 6h of losartan potassium treatment [4]. The terminal half-life of losartan and its active metabolite EXP 3174 was found to be 2.12 h and 6 to 9 h respectively [5]. The acute treatment of losartan (20 µmol/kg, i.p) significantly decreased Ang II-induced drinking behaviour at 4, 12 and 24h whereas its active metabolite, EXP 3174 could able to reduce water intake even at 0.25 h [6]. These findings suggested that EXP 3174 might be responsible for central actions of losartan [6]. Exp 3174 was found to be 20 times more potent than losartan in inhibiting central AT_1 receptors by easily crossing the blood-brain barrier [6].

The present study is intended to explore further the importance of the active metabolite of losartan potassium for its CNS activity using a mouse model of forced swim test, a test for potential antidepressant activity. It is therefore designed to evaluate the effect of losartan potassium on the immobility time in the forced swim test using rifampicin (cytochrome P450 enzyme inducer) and fluconazole (cytochrome P450 enzyme inhibitor) treated mice respectively.

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MATERIALS AND METHODS

Male swiss albino mice (20-25 g) were used in the study. Animals were allowed food and water *ad*

libitum up to the time of experimentation. The mice were housed in polycarbonate cages in groups of six animals under natural light-dark cycle. Institutional Animal Ethics Committee of BITS-Pilani approved the research protocol (protocol Number: IAEC/RES/5). Losartan potassium (Sun Pharma, India) was dissolved in normal saline and administered orally in a constant volume of 1 ml per 100 g of body weight. Rifampicin (Fourrts Laboratories, India), and fluconazole (Cipla Ltd, India) were prepared in 0.5%w/v of sodium carboxymethyl cellulose (CMC) and administered orally using oral feeding needle. All the drug solutions were prepared afresh at the beginning of each experiment. In rifampicin-treated group, rifampicin (300 mg/kg, p.o.) was administered for 10 days, whereas in fluconazole-treated group, fluconazole (30 mg/kg, p.o.) was administered 1h prior to losartan potassium treatment. The cytochrome P450 enzyme induction and inhibition doses of rifampicin and fluconazole respectively, were chosen based on the literature reported earlier [7, 8].

The behavioural despair test, commonly known as Porsolt forced swimming test is used to screen antidepressants [9]. This test consists of two trials. In the first trial on the first day, the animals were forced to swim individually in a glass cylinder (30 cm high, 22.5 cm in diameter) containing water up to 15 cm for 15 min at a room temperature. On the second day, the second trial (test) was carried out for 6 min. During 6 min test, each animal was placed on the cylinder one at a time and the duration of immobility was recorded by a blind, trained observer for this study. The animal was assessed as immobile when it stopped struggling and remained floating motionless in the water with slow pedalling necessary to keep its head above water [9, 10].

RESULTS

The effect of losartan potassium on forced swimming-induced immobility in rifampicin and fluconazole pre-treated mice respectively is shown in Table 1. In vehicle-treated group, losartan potassium (20 mg/kg, p.o) *per se* significantly enhanced the immobility time at 3h and 6h in the forced swim test. In rifampicin-treated group, losartan potassium treatment (20 mg/kg, p.o) showed a significant enhancement in immobility even at 1 h. However, in fluconazole-treated group, none of the time points after treatment of losartan potassium had shown any significant changes in immobility time (Table 1). Interestingly, it was also observed that the immobility time of rifampicin-treated control group exhibited a significant ($p<0.001$) reduction in the immobility time when compared with vehicle-treated control group (Table 1).

Table 1- Effect of losartan potassium (LP) on immobility in rifampicin and fluconazole-treated mice respectively using forced swimming test in mice (6 min test).

Treatment (mg/kg, p.o)	Immobility time (s)	ANOVA values
Vehicle treated groups		
Control	256.83 ± 4.10	F (3,20) = 18.045
LP (20)[1h prior]	261.32 ± 5.92	p < 0.0001
LP (20) [3h prior]	304.70 ± 7.43**	
LP (20) [6h prior]	309.02 ± 7.94**	
Rifampicin (300) treated groups		
Control	60.49 ± 12.28†	F (3,20) = 29.705
LP (20) [1h prior]	189.82 ±19.74**	p < 0.0001
LP (20) [3h prior]	278.44 ± 7.42**	
LP (20) [6h prior]	208.42 ±22.75**	
Fluconazole (30) treated groups		
Control	239.85 ± 19.03	F (3,20) = 0.0790
LP (20) [1h prior]	254.83 ± 21.18	p = 0.9706
LP (20) [3h prior]	249.60 ± 28.70	
LP (20) [6h prior]	244.60 ± 21.68	

Values are means ± SEM of 6 animals in each group. **p <0.01 (one-way ANOVA/ Dunnett's test; as compared with corresponding control group). † p <0.001 when compared with vehicle-control group.

DISCUSSION

In our previous studies, losartan potassium exhibited biphasic effects on immobility in mice (i.e.) it reduced immobility at lower doses (0.1, 1 and 5 mg/kg) and enhanced immobility at a higher dose level (100 mg/kg, i.p). Losartan potassium at a dose of 20 mg/kg, i.p. showed a significant enhancement in immobility at 3 h and suggested a possibility of the involvement of the active metabolite, EXP 3174 for its depressant-like activity in mice [4]. The importance of the active metabolite, EXP 3174 in the forced swim test is further confirmed in the present study of the interaction of losartan potassium with cytochrome P450 enzyme inducer (rifampicin) and inhibitor (fluconazole). *In vitro* experiments with human liver microsomes and specific inhibitors of different CYP1 enzymes indicated a role for CYP2C9 [11] and CYP3A4 [12] in the metabolism (oxidation) of losartan. Furthermore, *in vivo* studies revealed that fluconazole, an inhibitor of both CYP2C9 and CYP3A4, blocked the metabolism of losartan to EXP 3174 [13, 14]. In other studies, a strong induction of cytochrome P450 3A-dependent enzyme activities were observed in female rat liver microsomes after high dose treatment ($\geq$ 250 mg/kg/day for 9 days) with rifampicin [7].

Losartan potassium when administered with rifampicin-treated mice, showed an augmentation in immobility time even at 1 h. This might be due to an enhancement in the rate of metabolic process of losartan potassium by cytochrome P450 enzyme induction, which leads to an increased bioavailability of the active metabolite, EXP 3174. Surprisingly, the control immobility time of rifampicin *per se*-treated animal was significantly lower when compared with the vehicle-control. These findings raised the question that whether rifampicin *per se* possess antidepressant-like property or CYP enzyme induction leads to antidepressant activity. However, further preclinical and clinical studies are called for to confirm the antidepressant-like effect of rifampicin *per se*. But with fluconazole-treated mice, none of the time points after losartan potassium treatment had shown any significant changes in immobility time. Since, fluconazole might completely block the metabolism of losartan leading to an absence of formation of the active metabolite, EXP 3174. These results suggested that the depression-like effect of losartan potassium might be mainly mediated by its active metabolite, EXP 3174. The present results are consistent with our earlier findings reported that neuromodulatory effect of losartan potassium on dopaminergic system in mice was mainly mediated by its active metabolite EXP 3174 [15]. It was also reported that EXP 3174 could cross the blood-brain barrier more effectively than losartan despite the marked differences in their distribution ratios for octanol/water [6]. However, further research is warranted to find out whether active metabolite of losartan, EXP 3174 *per se* can contribute the central pharmacological actions which can be utilized in the treatment of various CNS disorders. Recently telmisartan, an angiotensin AT_1 receptor blocker has been reported for a significant bell shaped dose-dependent depression- like effect in the forced swim test [10]. It has been suggested that the depression- like effect produced by telmisartan might be due to the activation of the AT_2 receptors, since AT_1 receptor antagonist specifically block the effect of ang II on the AT_1 receptor and redirect the effects of ang II to the unopposed AT_2 receptor. Therefore, stimulation of AT_2 receptor as an indirect result of AT_1 receptor antagonism may contribute to the depression-like effect of telmisartan [10]. The similar mechanism of action might be responsible for depression-like effect of losartan at higher doses. In current clinical settings, losartan is well tolerated by patients with no major adverse effects including depression. These discrepancies might be due to species and dose differences. The dose at which losartan potassium has shown depression-like activity in mice is far greater than the human therapeutic dose.

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REFERENCES

[1] JW Wright, JW Harding. Brain renin-angiotensin-A new look at an old system. Prog Neurobiol.; 95: 49-67 (2011).

[2] M Bader, D Ganten, It's Renin in the Brain: Transgenic Animals Elucidate the Brain Renin-Angiotensin. Circ Res. 90: 8-10 (2002).

[3] D Ganten, K Hermann, C Bayer, T Unger, RE Lang. Angiotensin synthesis in the brain and increased turnover in hypertensive rats. Science. 221: 869-71 (1983).

[4] P Vijayapandi, AN Nagappa. Biphasic effects of losartan potassium on immobility in mice, Yakugaku Zassi. 125: 653-57 (2005).

[5] A Munafo, Y Christen, J Nussberger, LY Shum, MR Borland, RJ Lee, B Waeber, J Biollaz, HR Brunner. Drug concentration response relationships in normal volunteers after oral administration of losartan (DuP 753, MK 954), an angiotensin II receptor antagonist. Clin Pharmacol Ther. 51: 513-21(1992).

[6] C Polidori, R Ciccocioppo, P Pompei, R Cirillo, M Massi. Functional evidence for the ability of angiotensin AT1 receptor antagonists to cross the blood–brain barrier in rats. Eur J Pharmacol. 307: 259-67(1996).

[7] F Oesch, M Arand, MS Benedetti, MG Castelli, P Dostert. Inducing properties of rifampicin and rifabutin for selected enzyme activities of the cytochrome P-450 and UDP-glucuronosyltransferase superfamilies in female rat liver. J Antimicrobial Chemotherapy.37: 1111–19 (1996).

[8] I LaDelfa, YF Xia, TF Blaschke. Dose-dependent inhibition of cyclosporine metabolism in mice by fluconazole. Can J Physiol Pharmacol. 68: 89-93(1990).

[9] RD Porsolt, A Bertin, M Jalfre. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther.; 229:327-36 (1977).

[10] P Vijayapandi, S Harisankar, J Nancy. Depression- like effect of telmisartan in mice forced swim test: Involvement of brain monoaminergic system. J Pharmacol Toxicol. 7: 87-95 (2012).

[11] RA Stearns, PK Chakravarty, R Chen, SH Chiu. Biotransformation of losartan to its active carboxylic acid metabolite in human liver microsomes. Role of cytochrome P4502C and 3A subfamily members. Drug Metab Dispos. 23: 207-15 (1995).

[12] CH Yun, HS Lee, H Lee, JK Rho, HG Jeong, FP Guengerich. Oxidation of the angiotensin II receptor antagonist losartan (DuP 753) in human liver microsomes. Role of cytochrome P4503A(4) in formation of the active metabolite EXP3174. Drug Metab Dispos. 23: 285–89 (1995).

[13] KM Kaukonen, KT Olkkola, PJ Neuvonen. Fluconazole but not itraconazole decreases the metabolism of losartan to E-3174. Eur J Clin Pharmacol.; 53: 445-9 (1998).

[14] DJ Kazierad, DE Martin, RA Blum, DM Tenero, B Ilson, SC Boike, R Etheredge, DK Jorkasky. Effect of fluconazole on the pharmacokinetics of eprosartan and losartan in healthy male volunteers. Clin Pharmacol Ther. 62: 417-25 (1997).

[15] V Pandi, AN Nagappa, Thakurdesai PA, Effects of losartan potassium on central dopaminergic system in mice, J Pharmacol Toxicol.2: 551-58 (2007).