Research Article

Possible influence of Loxoprofen in lipopolysaccharide induced alterations in immobility-time in tail suspension test in mice

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Received: 2 January 2021 Revised: 24 February 2021 Accepted: 27 February 2021

Abstract

Background: It has been reported that there is an abnormal prostaglandin E levels in depression. Various studies indicated that there has been an elevated levels of prostaglandins (PGs) specifically prostaglandin E_2 (PGE_2) in depression. **Objective:** The objective of the present study was to evaluate the influence of Loxoprofen on immobility time in absence or presence of Lipopolysaccharide in tail suspension test model of depression in mice. **Material and Methods:** There was a measurement of immobility time in tail suspension test (TST) in which mice were subjected to the treatment of Loxoprofen (16.8 mg/kg, *p.o.*). The same drug treatment was given 60 min before Lipopolysaccharide [LPS](0.5 mg/kg, *i.p.*) and 23 h following LPS separately in mice. There was an evaluation of effect of above mentioned drugs in locomotor activity of mice. **Results:** The result of the present study indicated that mice treated with Loxoprofen showed a significant decrease in the immobility time in tail suspension test. LPS-treated mice presented an increase in immobility time when compared to controls 24 h after LPS administration. Similarly, Loxoprofen could only reverse but did not prevent the LPS-induced alterations in the TST. There was no significant effect of Loxoprofen on locomotor activity in mice. **Conclusion:** The results of the present study indicated that Loxoprofen could influence LPS induced alterations in immobility time in mice in tail suspension test. It also indicated possible anti-depressant effect of Loxoprofen in mice subjected to tail suspension test model of depression, having its possible implication in future treatment of depression.

***Keywords*:** Depression, tail suspension test, lipopolysaccharide, Loxoprofen

Introduction

Depression is a common mood disorder of mental illness, with the main clinical symptoms of abnormal behaviours and low spirits. It has been reported from that by 2020 depression may begin to be the principal cause of all non-fatal diseases. Furthermore, it may also become the second-most disabling disease after cardiac disease (Simon, 2003).

Various theories have been described for the aetiology of depression. One of the theories is the deficiency of noradrenaline and serotonin in certain brain which may be the underlying neurobiological mechanism of depression (Schildkraut, 1965; Taylor *et al*., 2005). There is still a certain amount of patients that either have not achieved remission or may initially respond but fail to maintain and the rest others may be non-responders (Stahl, 2000; Thase, 2001). Although effective, traditional antidepressants most often produce partial symptomatic improvement rather than symptom resolution and remission (Farvolden *et al*., 2003). In addition, current antidepressants are effective; they still have a lag time to onset of clinical response and are associated with many side effects (Farvolden *et al*., 2003).

In the light of above reports of the limitations of current antidepressants, there is a need of extensive research for finding the effective drugs based on novel pathophysiology and targets in depression. Likewise, the role of inflammation and inflammatory mediators in the neurobiology of depression has received a considerable amount of research attention in the past few years (Farooq *et al*., 2017; Felger and Lotrich, 2013; Patel, 2013; Jangpangi, 2016; Noto *et al*., 2014). It has been reported that there is an abnormal prostaglandin levels in depression (Calabrese *et al*., 1986; Lieb *et al*., 1983; Linnoila *et al*., 1983). Several studies indicated that there have been elevated levels of

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DOI: https://doi.org/10.31024/ajpp.2021.7.1.4

prostaglandins (PGs) in depression (Calabrese *et al*., 1986; Fritz *et al*., 2016; Lieb *et al*., 1983)

Further, neuro-inflammation may be contributed by PGE_2 as concluded from various preclinical studies (Brenneis *et al*., 2010). Several reports has shown that there has been increase in levels of inflammatory mediators, prostaglandin in the saliva, plasma and CSF of depressed individuals (Calabrese *et al*., 1986; Lieb *et al*., 1983; Linnoila *et al*., 1983; Nishino *et al*., 1989; Ohishi *et al*., 1988). These findings were found to have a relation to the severity of depression (Nishino *et al*., 1989; Ohishi *et al*., 1988). Signs of an inflammatory process particularly high prostaglandin levels, have repeatedly been described in major depression (De Paiva *et al*., 2010). Further, it has been reported that cyclooxegenase-2 (COX-2) up-regulation is associated with increased PGE_2 level and neuronal apoptosis (Li *et al*., 2003). COX-2 inhibitors inhibited the PGE_2, suggesting a positive role in depression (Müller *et al*., 2003). Furthermore, several reports suggested that several antidepressant drugs could inhibit PGE_2 synthesis (Lee, 1974).

Loxoprofen is a drug of non-steroidal anti-inflammatory drugs category, having anti-inflammatory properties. In several preclinical studies reports, it has been found that Loxoprofen remarkably decreased the PGE_2 level in regions of brain (Brenneis *et al*., 2010). Considering above mentioned evidences, reports and property of inhibition of PGE_2 of Loxoprofen, it can be hypothesized that Loxoprofen may have effect on the depressive behaviours in experimental animals. Hence, it has been proposed to study the effect of Loxoprofen in various animal models of depression in mice.

Material and methods

Animals

Swiss albino male mice were obtained from Zydus Research Centre, Moraiya and Ahmedabad. They were housed under standard condition with free access to food and water, under 12:12 hr light: dark cycle. Mice were allowed to acclimatize for 07 days before the initiation of behavioural tests were performed out. Each animal was used only once (N= 6 animals per group). The experiments were performed after the protocol for experimental design were approved with protocol no. BIP/IAEC/2018/06 by the Institutional Animal Ethics Committee (IAEC) of Babaria Institute of Pharmacy. The experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA).

Drugs and dosing detail

Loxoprofen [LOX] was administered in the dose of 16.8 mg/kg, *p.o.* (Futaki *et al*., 2009; Ghosh, 2008). The report from the study of futaki and their co-workers indicated that Loxoprofen could significantly reduce PGE_2 levels in cerebrospinal fluid in rats in the dose of 2.4 mg/kg. The same dose of 2.4 mg/kg of rats has been converted into mice dose as per previously reported method (Ghosh, 2008), considering 0.14 as surface area ratio for 20 gm of mice which was then ultimately converted into dose per kg, giving the dose of 16.8 mg/kg in mice. Fluoxetine [FLX] as selective serotonin reuptake inhibitor was administered in the dose of 20 mg/kg *p.o.* (Tayal *et al*., 2008). Bupropion [BUP] as selective dopamine reuptake inhibitor was administered in the dose of 20 mg/kg *i.p.* (Dhir and Kulkarni, 2007). Atomoxetine [ATM] as selective noradrenaline reuptake inhibitor was be administered in the dose of 1 mg/kg *i.p.* (Koda *et al*., 2010). Venlafaxine [VEN] as triple monoamine reuptake inhibitor was administered in the dose of 4 mg/kg *i.p.* (Thomas *et al*., 2016). Lipopolysaccharide [LPS] was administered in the dose of 0.5mg/kg *i.p* (Mello *et al*., 2013). Loxoprofen was dissolved in 0.5 % CMC solution (Futaki *et al*., 2009). Fluoxetine, Bupropion, Atomoxetine and Venlafaxine were dissolved in normal saline solution (0.9% NaCl) (Dhir and Kulkarni, 2007; Koda *et al*., 2010; Tayal *et al*., 2003; Thomas *et al*., 2016). LPS was dissolved in phosphate buffered saline (PBS) solution (Mello *et al*., 2013).

Tail Suspension Test (TST) method

Groups of animals

For experiment in tail suspension test, mice were divided into following groups (N=6) each as follows: Group I received saline and served as Vehicle Control- I (VC-I), Group II received 0.5% CMC served as Vehicle Control- II (VC-II), Group III received Fluoxetine (20 mg/kg *p.o)*, Group IV received Atomoxetine (1 mg/kg *i.p*), Group V received Bupropion (20 mg/kg *i.p*), Group VI received Loxprofen (16.8 mg/kg *p.o*), Group VII - I & II received Lipopolysaccharide (0.5mg/kg *i.p.*), Group VIII - I & II received Lipopolysaccaride (0.5mg/kg *i.p.*) along with Venlafaxine (4 mg/kg *i.p*), Group IX - I & II received Lipopolysaccharide (0.5mg/kg *i.p.*) along with Loxoprofen (16.8 mg/kg *p.o.*).

Animal Protocol and treatment schedule

In this set of experiment, mice were given the therapy of above mentioned previously reported doses of drugs. On day of dosing, all mice were then subjected to tail suspension test after 60 minutes as per last dose of oral administration or after 30 minutes as per last dose of intra-peritoneal administration.

For the pre-treatment paradigm, mice of group VII-I received only LPS (0.5 mg/ kg, *i.p.*) administration. Mice of group VIII-I received Venlafaxine (4 mg/kg, *i.p*) 30 min prior to LPS (0.5 mg/ kg, *i.p.*) administration. Mice of group

IX-I received Loxoprofen (16.8 mg/kg, *p.o*) 30 min prior to LPS (0.5 mg/ kg, *i.p.*) administration (Mello et al., 2013).

For the post-treatment protocol, separate animals were used. Mice of group VII-II received only LPS (0.5 mg/ kg, *i.p.*) administration. Mice of group VIII-II received Venlafaxine (4 mg/kg, *i.p*) 23.5 min after LPS (0.5 mg/ kg, *i.p.*) administration. Mice of group IX-II received Loxoprofen (16.8 mg/kg, *p.o*) 23.5 after LPS (0.5 mg/ kg, *i.p.*) administration (Mello et al., 2013). Immobility time was measured in both situations (pre- and post-treatment) 24 h after LPS administration (Mello et al., 2013).

Mice were suspended on the edge of a table 58 cm above the floor by the adhesive tape placed approximately 1cm from the tip of the tail. Immobility time was recorded during a 5 min period. The animal was regarded as immobile if the animal doesn't show any movement of body and hanged passively. Evaluation parameter was immobility time (Vogel, 2002).

Loco-motor activity (LMA)

Animal grouping

For experiment in evaluating the loco-motor activity, mice were divided into total six groups (N=6) each as follows: Group I received saline and served as Vehicle Control- I (VC-I), Group II received 0.5% CMC served as Vehicle Control- II (VC-II), Group III received Fluoxetine (20 mg/kg *p.o.)*, Group IV received Atomoxetine (1 mg/kg *i.p.*), Group V received Bupropion (20 mg/kg *i.p.*), Group VI received Loxoprofen (16.8 mg/kg *p.o.*).

Treatment schedule of drugs and paradigm followed in LMA

In this set of experiment, mice were given therapy of above mentioned previously reported single dose of drugs. All animals were then subjected to locomotor activity test using a photoactometer for five minutes 60 minutes as per last dose of oral administration or after 30 minutes as per last dose of intraperitoneal administration. Evaluation parameter was the counts on photoactometer (Vogel, 2002).

Statistical analysis

All quantified data were expressed as mean ± S.E.M. for the indicated analyses. Statistical comparisons were performed by one-way ANOVA followed by the Tukey's Test. $P < 0.05$ were considered as significant. All statistical analysis were performed using approved statistical software (Sigmastat software, Systat Software Inc, San Jose, CA, USA.).

Results

Effect of Loxoprofen on immobility time in tail suspension test (TST) in absence of LPS

There was a significant decrease in immobility time in Fluoxetine, Bupropion and Atomoxetine treated mice as compared to vehicle control-I. Further, there was a significant decrease in immobility time in Loxoprofen treated mice as compared to vehicle control-II. Loxoprofen decreased the immobility time more as compared to the standard treatment in TST but no significant difference was observed between the groups (Table 1).

Effect of Loxoprofen on immobility time in tail suspension test (TST) in pre-treatment groups of LPS

In the pre-treatment groups of LPS, Venlafaxine decreased the immobility time as compared to LPS only while no significant changes were observed in Loxoprofen treated animals as compared to LPS only (Table 2).

Effect of Loxoprofen on immobility time in tail suspension test (TST) in post-treatment groups of LPS

In the post treatment groups of LPS, both Venlafaxine and Loxoprofen decreased the immobility time as compared to only LPS treated group. Also in post treatment groups of LPS, Loxoprofen decreased the immobility time less as compared to Venlafaxine in TST but no significant difference was observed between the groups (Table 3).

Effect of Loxoprofen on the number of counts in Locomotor activity test

There was no significant change in counts in Fluoxetine, Bupropion and Atomoxetine treated mice as compared to vehicle control-I. There was no significant change in

Table 1. Effect of Loxoprofen on immobility time in tail suspension test (TST) in absence of LPS

Group No.	Treatment	Dose (mg/kg)	Route	Immobility time (secs) Mean ± SEM
I	Vehicle Control I (VC I)	10 mg/kg	*p.o*	215.667±12.598
II	Vehicle Control II (VC II)	10ml/kg	*p.o*	224.833±10.349
III	Fluoxetine	20 mg/kg	*p.o*	172.000 ±14.960*
IV	Bupropion	20mg/kg	*i.p*	154.833 ± 5.121*
V	Atomoxetine	1 mg/kg	*i.p*	179.000 ± 6.643*
VI	Loxoprofen	16.8 mg/kg	*p.o*	169.000 ± 12.546*

Each column expressed as Mean ± SEM of 6 animals after respective treatments. Data were analysed by One Way Analysis of Variance (ANOVA) followed by Tukey's test. *P<0.05 when compared with VC-I, $^{+}$ p < 0.05 when compared with VC-II

Table 2. Effect of Loxoprofen on immobility time in tail suspension test (TST) in pre-treatment groups of LPS

Group No.	Treatment	Dose (mg/kg)	Route	Immobility time (secs) Mean ± SEM
VII-I	LPS	0.5 mg/kg	*i.p.*	202.000 ± 4.604
VIII-I	LPS + Venlafaxine	0.5 mg/kg + 4 mg/kg	*i.p.*	184.333 ± 4.387$^{\#}$
IX-I	LPS + Loxoprofen	0.5 mg/kg + 16.8 mg/kg	*i.p.* &*p.o.*	196.167 ± 6.183

Each column expressed as Mean ± SEM of 6 animals after respective treatments. Data were analysed by One Way Analysis of Variance (ANOVA) followed by Tukey's test. $^{\#}P<0.05$ when compared with group VI-I

Table 3. Effect of Loxoprofen on immobility time in tail suspension test (TST) in post-treatment groups of LPS

Group No.	Treatment	Dose (mg/kg)	Route	Immobility time (secs) Mean ± SEM
VII-II	LPS	0.5 mg/kg	*i.p.*	200.000 ± 7.668
VIII-II	LPS + Venlafaxine	0.5 mg/kg + 4mg/kg	*i.p.*	166.000 ± 13.013$^{@}$
IX-II	LPS + Loxoprofen	0.5 mg/kg + 4mg/kg	*i.p.* & *p.o.*	158.667 ± 12.379$^{@}$

Each column expressed as Mean ± SEM of 6 animals after respective treatments. Data were analysed by One Way Analysis of Variance (ANOVA) followed by Tukey's test. $^{@}P<0.05$ when compared with VI-II

Table 4. Effect of Loxoprofen on the no of counts in Locomotor Activity Test

Group No.	Treatment	Dose (mg/kg)	Route	No of Counts Mean ± SEM
I	Vehicle Control I (VC I)	10 mg/kg	*p.o.*	64.333 ± 20.575
II	Vehicle Control II(VC II)	10ml/kg	*p.o.*	74.667 ± 13.817
III	Fluoxetine	20 mg/kg	*p.o.*	78.833 ± 3.20.387
IV	Bupropion	20mg/kg	*i.p.*	72.167 ± 3.928
V	Atomoxetine	1 mg/kg	*i.p.*	44.833 ± 8.538
VI	Loxoprofen	16.8 mg/kg	*p.o.*	76.833 ± 12.986

Each column expressed as mean ± SEM of 6 animals after respective treatments . Data were analysed by One Way Analysis of Variance (ANOVA) followed by Tukey's test.

immobility time in Loxoprofen treated mice as compared to vehicle control-II (Table 4).

Discussion

The results of the present investigations indicated the influence of Loxoprofen on Lipopolysaccaride induced alterations in immobility time in tail suspension test in mice. It also indicated the possible antidepressant like effect of Loxoprofen in acute model of depression in mice. While the conclusion, derived from locomotor activity showed that Loxoprofen did not affect the central nervous system either in terms of its stimulation or inhibition. Loxoprofen decreased the immobility time in tail suspension test.

It has been reported that various NSAIDS drugs have shown antidepressant like effect in various experimental animal models (Santiago el al., 2014; Zaminelli et al., 2014). However, the mechanism by which Loxoprofen indicated a significant anti-depressant action in tail suspension test remains to be elucidated. Though, the previously reported inhibitory action of Loxoprofen on PGE_2 synthesis may show potential role of PGE_2 in mediating the anti-depressant effect of Loxoprofen in animal models of depression. It can also be possible that antidepressant effect of Loxoprofen at specified dose as mentioned in the present study may be achieved by the inhibition of PGE_2 in brain. It is also possible that such Loxoprofen induced PGE_2 inhibition may be responsible for the inhibition of synthesis of inflammatory mediators. The present results may indicate that prostaglandins synthesis may be necessary for the development of depressive-like and exploratory behaviours in mice and Loxoprofen being PGE_2 inhibitor in brain and also being COX inhibitors eliminate this reaction and decreased LPS-induced behaviours while it doesn't prevent the LPS-induced behaviours.

Although we were unable to measure brain PGE_2 levels in brain but further research work is suggested to examine if Loxoprofen induced alteration in brain PGE_2 levels may affect the molecular mechanism of depression.

The behaviour sampling data of tail suspension test shows the potential anti-depressant action of Loxoprofen in mice, but further work is required for further exploration of the present investigations.

Regardless any previous reports or studies, these are the first results for possible influence of Loxoprofen on LPS induced alterations in immobility time in tail suspension test and the possible anti-depressant like effect of Loxoprofen in tail suspension test model of depression in mice, having its further potential implication in the pathophysiology and treatment of depression in future.

Conclusions

The results of the present study indicated that Loxoprofen could influence LPS induced alterations in immobility time in mice in tail suspension test. It also indicated the possible anti-depressant effect of Loxoprofen in mice subjected to tail suspension test model of depression, having its possible implication in future treatment of depression.

Acknowledgements

The authors are grateful management team of Babaria Institute of Pharmacy, Vadodara, India for providing necessary facilities for this work. Also thankful to Zydus Research Centre, Ahmedabad for providing animals and all other pharmaceutical companies for providing gratis sample of drug for this research.

Conflict of interest

The authors declare no conflicts of interest.

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