Effect of 5-lipoxygenase inhibitor against lipopolysaccharide-induced hypothermia in mice

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Bacterial endotoxin produces sepsis associated with alterations in body temperature (fever or hypothermia). The intraperitoneal administration of bacterial endotoxin, lipopolysaccharide (LPS; 50 μg/mouse) led to a decrease in colonic temperature starting 1 hr after the injection. The hypothermic effect was accompanied by a significant increase in hypothalamic leukotriene B4 (LTB4) and prostaglandin E2 (PGE2) levels. 5-lipoxygenase inhibitor, zileuton (200 and 400 mg/kg, po) administered 30 min before LPS challenge significantly prevented hypothermia. However, non-selective cyclooxygenase inhibitor, indomethacin (10, 20 mg/kg, po) did not reverse the hypothermic response. Further, pretreatment of mice with zileuton prevented LPS-stimulated increase in hypothalamic LTB4 levels and caused a relatively small increase in PGE2 levels. Indomethacin had no effect on LTB4 levels but it reduced PGE2 levels. These results suggest a possible involvement of leukotrienes in LPS-induced hypothermia and the potential protective role of 5-lipoxygenase inhibitors in endotoxemia.

Keywords: Hypothermia, Lipopolysaccharide, Leukotriene B4, 5-Lipoxygenase, 5-LOX, Prostaglandin E2.
leukotrienes in mediating the endotoxin-induced hypothermia is suggested. The present study has been aimed to evaluate the role of LTs in LPS-induced hypothermia and protective use of 5-lipoxygenase (5-LOX) inhibitor in mice. The effect of this inhibitor on the brain leukotriene levels was also estimated.

Materials and Methods

Animals — Swiss mice, 25-30 g (Central Animal House, Panacea Biotec Ltd., Lalru) of either sex were used. They were housed in plastic cages at ambient room temperature (24±0.5°C) and 12 hr:12 hr light:dark cycle. Ten mice were used per treatment group. Animals were given food and water ad libitum. Experiments were carried out between 0900 and 1500 hrs. The experimental protocols were approved by the Institutional Animal Ethics Committee.

Drugs and regimen — Zileuton, (Archechem, Mumbai India), indomethacin (Panacea Biotec Ltd., Lalru, India), and Lipopolysaccharide (LPS) from Salmonella typhosa (Sigma USA), were used. LPS was dissolved in saline and injected at a dose of 50 μg/mouse. All drugs were suspended in Tween 80 and administered perorally in the dose volume of 10 ml/kg, 30 min before intraperitoneal administration of LPS.

Measurement of Leukotriene B4 levels in hypothalamus — Leukotriene B4 levels were estimated by modified method of Singh et al. Mice were sacrificed by decapitation at 4 hr after LPS administration and the whole hypothalamus was quickly excised by tweezers under cold conditions and stored at -13°C in potassium chloride (KCl; 1.15% w/v) buffer till further estimation. The landmarks of hypothalamus were defined according to Franklin and Paxinos: the mouse hypothalamus is a formation located on the ventral behind the optical chiasmus and is visibly distinct from surrounding tissues by color (grey) and slightly prominent margins. The individual isolated hypothalamus was weighed and homogenized in KCl 1.15% solution (1 ml:0.9 g tissue weight). The homogenate was centrifuged (-13°C, 11,000 rpm, 20 min), and supernatant separated. The LTB4 contents were determined using LCMS/MS (facility at Panacea Biotec ltd., Lalru: API 3000; PE SCIEX, using turbo ion spray as ion source. Column C18/5cm/Novopak, mobile phase: 0.001% ammonium acetate and acetonitrile in the ratio 65:35, flow rate 0.2 ml/min, injection volume: 50 μl). A linearity upto 500 pg concentration was observed.

Authentic standard of LTB4 (Sigma USA) was used to identify the LTB4 peak and octacosanol was used as an internal standard (leukotriene IS).

Results

Effect of LPS on colonic temperature — Mice injected with LPS displayed a significant decrease in colonic temperature within 1 hr of post injection...
(pretreatment value: 37°±0.73°C; 1 hr post treatment value: 35.8°±0.2°C) as compared to the control group (pretreatment value: 37.21°±0.54°C; 1h post treatment value: 37.01°±0.2°C). The hypothermic response in LPS-treated mice was observed during the next 3 hr. The change in colonic temperature is shown in Fig. 1.

Effect of zileuton, and indomethacin on LPS-induced colonic temperature — Oral administration of zileuton (200, and 400 mg/kg, po) prevented hypothermic effect of LPS in mice (P=0.005, F=14.926, MANOVA) (Fig. 2a). Further, indomethacin (10, 20 mg/kg, po) did not reverse the LPS-induced hypothermia in mice throughout the observation period (Fig. 2b).

Effect of various treatments on percent mortality after LPS treatment — All LPS treated mice died within 24 hr. Pretreatment with zileuton 100, 200 and 400 mg/kg resulted in mortality rate of 4/10, 3/10, 1/10 (dead/total), respectively. However, a mortality rate of 9/10 was observed for indomethacin (10, and 20 mg/kg).

Effect of zileuton, and indomethacin on hypothalamic LTB4 content — Hypothalamic LTB4 content was determined 4 hr post LPS injection. Intraperitoneal administration of LPS resulted in a significant (p<0.05) elevation of LTB4 content (18.57 ±1.5 ng/g tissue vs. 13.27 ±1.1 ng/g tissue of control). Zileuton (400 mg/kg, po) inhibited the elevation in hypothalamic LTB4 content due to LPS administration. Interestingly, indomethacin (20 mg/kg, po) showed a significant increase in LTB4 content as compared to LPS treated group (P=0.024, F=12.928, ANOVA) (Fig. 3).

![Fig. 1](image1.png)  
**Fig. 1** — Changes in body temperature of mice in response to intraperitoneal administration of lipopolysaccharide (LPS: 50 μg/mouse), or saline (equivalent volume, ip). Each point represents the mean change in colonic temperature ± SE (n = 10 in each group). LPS: lipopolysaccharide; CTL: saline control. *P<0.05 significantly different from control group.

![Fig. 2](image2.png)  
**Fig. 2** — Effect of (a) zileuton (100, 200, and 400 mg/kg, po) (b) indomethacin (10 and 20 mg/kg, po) administered 30 min before LPS; 50 μg/mouse, ip, on body temperature in mice. Each point represents absolute temperature as mean ± SE (n = 10 in each group). *<0.05 as compared to control group; *<0.05 as compared to LPS treated group. LPS: lipopolysaccharide, Ind: indomethacin, CTL: control. (P=0.005, F=14.926, MANOVA)

![Fig. 3](image3.png)  
**Fig. 3** — HPLC chromatogram of PGE2.
Effect of zileuton, and indomethacin on hypothalamic PGE\textsubscript{2} content — PGE\textsubscript{2} levels were estimated in the hypothalamus after 4 hr. Fig. 4 represents HPLC chromatogram for PGE\textsubscript{2}. The peak area for PGE\textsubscript{2} following different treatment was compared. The PGE\textsubscript{2} levels were increased by 1.5 times with LPS treatment (272.9±10.2 ng/g tissue vs. 170.31±21.28 ng/g tissue of control). Pretreatment of mice with zileuton led to a relatively small elevation in PGE\textsubscript{2} content than in mice injected with LPS alone (Fig. 5). Further, indomethacin (20 mg/kg, po) treatment caused a marked decrease in PGE\textsubscript{2} content as compared to LPS-treated group (P<0.001, F=8.516, ANOVA) (Fig. 5).

Discussion

Bacterial endotoxin (LPS) induces a variety of metabolic, cellular and regulatory effects known as acute phase response (APR). One of the most prominent manifestations of APR is thermoregulatory alteration (fever or hypothermia). Thermoregulatory changes in response to LPS administration are dependent upon the surrounding ambient temperature. Derijk et al.\textsuperscript{24} reported hypothermia with LPS at subthermoneutral temperature of 24°C and fever at thermoneutral temperature of 30°C. The mechanisms involved in hypothermic response to endotoxin are not well understood.

In the present study LPS administration to mice maintained at subthermoneutral temperature (24°C) induced hypothermia, which was accompanied by an increase of hypothalamic LTB\textsubscript{4} content. Pretreatment with zileuton also reduced the mortality rate. Zileuton, a 5-LOX enzyme inhibitor, that inhibits the synthesis of LTs prevented the development of LPS-induced hypothermia and reversed the increased LTB\textsubscript{4} content. It therefore, seems that leukotrienes may induce hypothermic response to endotoxins. The maintenance of body temperature involves a balance between thermogenesis (heat production) and heat loss mechanisms (mainly vascular tone)\textsuperscript{12}, and if it is believed that LTs do possess a cryogenic property then the question arises: how do LTs exert the hypothermic effect and what is the probable mechanism of action of zileuton; peripherally or centrally?

LTs are known to produce vasoconstriction in majority of vascular beds\textsuperscript{25,26}. There is no evidence in regard to possible direct effect of LTs on temperature control through vascular tone\textsuperscript{9}. Hence, it seems skeptical that LTs promote heat loss. So, it is unlikely to consider role of peripheral vascular tone in reversal of LPS-induced hypothermia by zileuton. However, it may be possible that LTs released from peripheral macrophages gained access to hypothalamus and exert hypothermic effect. As demonstrated in the present study, LPS increased LTs content in hypothalamic, it may be presumed that the LTs released peripherally from macrophages may have gained access to hypothalamus and/or in combination with the local hypothalamic released LTs resulted in
stimulates LTs production, which may then act as endogenous cryogen. Further, indomethacin inhibits the hypothalamic effect in hypothalamus by inhibiting hypothalamic synthesis of LTs. In addition, LPS-induced change in thermal regulation is an early phase of immune-inflammatory cascade. Zileuton is reported to inhibit production of platelet activating factor and COA-IT, the major mediators of the early phase of immune-inflammatory episode.

Further, it was observed that, 5-LOX inhibitor, in parallel to reducing hypothalamic LTs also promoted LPS-stimulated PGE$_2$ levels in hypothalamus. Although feeble, this elevation in PGE$_2$ levels with 5-LOX inhibitor suggest a role of PGE$_2$ in their antihypothermic effect.

It was also observed that, pretreatment with indomethacin (a non-selective cyclooxygenase inhibitor) did not reverse the LPS-induced hypothermia in mice. The observation is in accordance with those of Kozak et al., who attributed this phenomenon to increased TNF-α bioactivity with indomethacin. TNF-α stimulates LTs production, which may then act as endogenous cryogen. Further, indomethacin increased hypothalamic LTs level. This, along with alteration of PGE$_2$ level by 5-LOX inhibitors suggest a role of PGE$_2$ in their antihypothermic effect.

Thus from the results of the present study it seems that leukotrienes play an important role in LPS-mediated hypothermia and could be considered as endogenous cryogens. However, several other mediators like prostaglandins, IL-10, TNF-α and vasopressin have been proposed to be involved in LPS-induced hypothermia. Yirmiya et al. reported the efficacy of chronic treatment of fluoxetine, a SSRI against LPS hypothermia. Reduction in vasopressin by fluoxetine seemed to be responsible for its antihypothermic effect. Nevertheless, the effects of antidepressants are probably mediated by a direct action on immune cells. Antidepressants were found to inhibit interleukin (IL), tumor necrosis factor-alpha (TNF-α) during LPS hypothermia. These immune mediators have been reported to stimulate the release of secondary mediators LTs by one or the other cascade. Thus it is also suggestive of the role of LTs in hypothermic response to endotoxin.

In conclusion, the results of the present study suggest the role of LTs in mediating endotoxin induced hypothermia and 5-LOX inhibitor like zileuton reversed LPS-induced hypothermia presumably by preventing the migration of non-hypothalamic LTs and inhibiting the hypothalamic release of LTs.

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References


