

# In vitro and in vivo modulatory effect of PBI-1393 on LPS-induced neutrophil activation and inflammatory response

Mustapha Allam, Nathalie Julien, Michel Asselin, Boulos Zacharie, Christopher Penney and Lyne Gagnon  
ProMetic BioSciences Inc., 500 Cartier Blvd. West, Suite 150, Laval, Quebec, Canada H7V 5B7



PROMETIC

## Abstract

Polymorphonuclear neutrophils (PMNs) play a key role in the inflammatory response against infectious agents. However, they can elicit significant tissue damage and in this respect, anti-inflammatory drugs are of interest. In this study, we examined the effect of PBI-1393, a low molecular weight immunomodulatory molecule, on PMN activation by LPS both *in vitro* and *in vivo*. The production of TNF- $\alpha$  by human LPS-activated PMN in the presence or absence of PBI-1393 was measured by ELISA. The ability of PBI-1393 to modulate PMN activation and recruitment *in vivo* was assessed using a rat air pouch model of inflammation. Exudates from controls and PBI-1393 treated animals ( $n=6$ ) were used to assess leukocyte infiltration and to measure (by ELISA) TNF- $\alpha$ , MCP-1 and PGE<sub>2</sub> production. *In vitro*, PBI-1393 is able to significantly decrease by 28.8%  $\pm$  0.08% ( $p<0.05$ ), TNF- $\alpha$  production by human LPS-activated PMN. *In vivo*, PBI-1393 significantly decreased the LPS-induced production of TNF- $\alpha$  by 41.4%  $\pm$  7.2% ( $p<0.005$ ), MCP-1 by 16.3%  $\pm$  8.4% ( $p<0.05$ ) and PGE<sub>2</sub> by 29.2%  $\pm$  13.5% ( $p<0.05$ ). However, PBI-1393 did not significantly inhibit leukocyte infiltration. These results show that PBI-1393 is able to modulate PMN activation and inflammatory response and suggest potential use as an anti-inflammatory agent.

## Aim

PBI-1393 is an immunostimulant able to modulate Th1 cytokine production by T lymphocytes. We sought to determine if this compound could also regulate neutrophil activation and subsequently modulate inflammatory response.

## Methods

### Human leukocyte isolation:

Human PMNs were isolated by lympholyte-poly (Cedarlane) according to manufacturer's protocol.

### Cell incubation:

PMNs were resuspended in at a concentration of  $5 \times 10^6$  cells/ml in Hank's balanced salt solution (HBSS; 37°C) containing 10 mM HEPES pH 7.4, 1.6 mM Ca<sup>2+</sup> and no Mg<sup>2+</sup>. Cells were treated with PBS or PBI-1393 10 min before stimulation with LPS (100 ng/ml). Cells were then incubated for 6 h and supernatants were harvested and stored at -20°C until analysis by ELISA.

### Determination of secreted TNF- $\alpha$ , PGE<sub>2</sub> and MCP-1 by ELISA:

Supernatants or exudates were harvested and clarified by centrifugation. Determination of cytokine or chemokine concentration was performed using commercially available specific ELISA kits, according to manufacturers' instructions.

### Dorsal air pouches:

Air pouches were raised on the dorsum of rats by subcutaneous injection of 20 ml of sterile air on day 0 and 10 ml on day 3. Experiments were conducted on day 6. One hour before LPS stimulation, animals were injected IV with saline or PBI-1393 (25 or 100 mg/kg). Individual air pouches (one per rat) were then injected either with 2.5 ml of endotoxin-free PBS alone or containing 500 ng LPS. Rats were sacrificed 12 h later and individual air pouches were washed two times with ice-cold PBS (total of 5 ml). Leukocyte suspensions were enumerated and their identification was assessed by Giemsa staining.

### Statistical analysis:

Where applicable, statistical analysis was performed by Student's *t* test (two-tailed) and significance (\*) was considered to be attained when  $p<0.05$ .

## Summary

- PBI-1393 decreased TNF- $\alpha$  production by human LPS-activated PMN *in vitro*.
- PBI-1393 had no significant effect on neutrophil infiltration.
- PBI-1393 decreased *in vivo* TNF- $\alpha$  production response to LPS stimulation.
- PBI-1393 decreased *in vivo* PGE<sub>2</sub> production in response to LPS stimulation.
- PBI-1393 decreased MCP-1 production in LPS-treated animals.

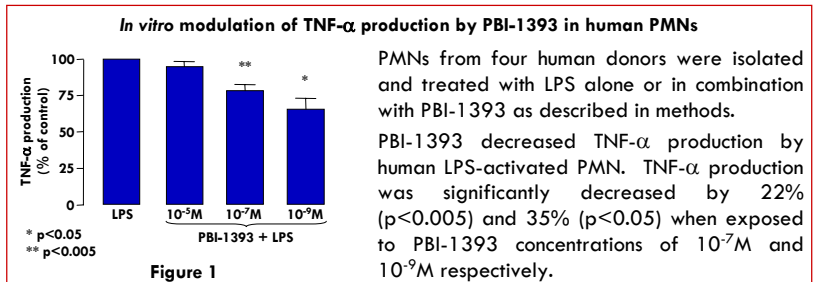


Figure 1

PMNs from four human donors were isolated and treated with LPS alone or in combination with PBI-1393 as described in methods. PBI-1393 decreased TNF- $\alpha$  production by human LPS-activated PMN. TNF- $\alpha$  production was significantly decreased by 22% ( $p<0.005$ ) and 35% ( $p<0.05$ ) when exposed to PBI-1393 concentrations of 10<sup>-7</sup>M and 10<sup>-9</sup>M respectively.

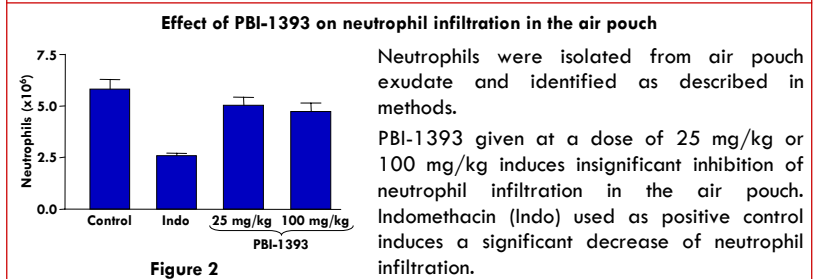


Figure 2

Neutrophils were isolated from air pouch exudate and identified as described in methods. PBI-1393 given at a dose of 25 mg/kg or 100 mg/kg induces insignificant inhibition of neutrophil infiltration in the air pouch. Indomethacin (Indo) used as positive control induces a significant decrease of neutrophil infiltration.

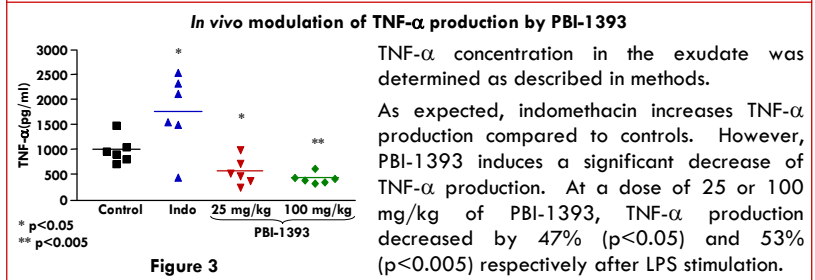


Figure 3

TNF- $\alpha$  concentration in the exudate was determined as described in methods. As expected, indomethacin increases TNF- $\alpha$  production compared to controls. However, PBI-1393 induces a significant decrease of TNF- $\alpha$  production. At a dose of 25 or 100 mg/kg of PBI-1393, TNF- $\alpha$  production decreased by 47% ( $p<0.05$ ) and 53% ( $p<0.005$ ) respectively after LPS stimulation.

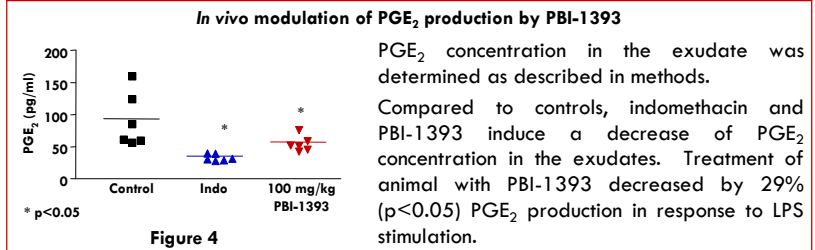


Figure 4

PGE<sub>2</sub> concentration in the exudate was determined as described in methods. Compared to controls, indomethacin and PBI-1393 induce a decrease of PGE<sub>2</sub> concentration in the exudates. Treatment of animal with PBI-1393 decreased by 29% ( $p<0.05$ ) PGE<sub>2</sub> production in response to LPS stimulation.

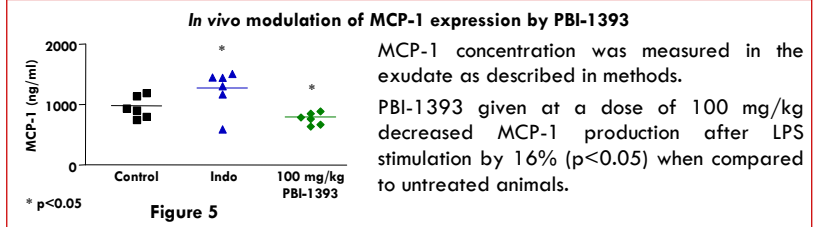


Figure 5

MCP-1 concentration was measured in the exudate as described in methods. PBI-1393 given at a dose of 100 mg/kg decreased MCP-1 production after LPS stimulation by 16% ( $p<0.05$ ) when compared to untreated animals.

## Conclusion

- PBI-1393 modulates the expression by neutrophils of inflammatory mediators in particular TNF- $\alpha$  and PGE<sub>2</sub> in response to an inflammatory stress.
- This effect could not be explained by the decrease of neutrophil number because PBI-1393 did not affect significantly the recruitment to these inflammatory cells. The result suggests the involvement of a second messenger shared by TNF- $\alpha$  and PGE<sub>2</sub> expression pathway.
- PBI-1393 is able to inhibit MCP-1 expression and subsequently it can modulate the recruitment of monocytes or macrophages to the site of inflammation.
- These results emphasize the potential clinical application of PBI-1393 as an anti-inflammatory drug.