Molecular Pharmacology (USA) Limited

Zn-plasma protein complex reduces cytokine production in LPS-stimulated monocytes

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INTRODUCTION

MPL is developing a biologically active anti-inflammatory agent, derived from fractionated bovine plasma proteins conjugated with zinc (Bov-Zn). Here, the ability of the proprietary Bov-Zn to regulate cytokine production by LPS-stimulated monocytes is assessed (exp.1). The results were validated by competitive inhibition studies between Bov-Zn and fetal calf serum (exp.2), and by assessment of the effect of Bov-Zn on monocyte metabolism (exp.3).

METHODS

For all experiments, human monocytes were cultured in RPMI/10% FCS at37ºC/5%CO^2.

Exp.1: Human monocyte cultures were stimulated with 500ng/ml LPS (E.coli 0111:B4) in the presence of 0%, 2.5%, 5%, 7.5%, 10%, 20% or 40% Bov-Zn. After 24 hrs incubation, culture media were collected, centrifuged, and assayed (TNF-*α* ELISA).

Exp.2: A competitive inhibition design was set up using 0%, 1%, 5%, 10% FCS against 0%, 2.5%, 5%, 10% Bov-Zn.

Exp.3: Metabolism of non-proliferating monocytes was measured via accumulation of bioreduced formazan (Promega CellTiter 96) in treated and untreated cell cultures over 0-45 hrs at intervals.

RESULTS

The IC_{50} for suppression of LPS-stimulated human monocyte TNF-α production was reached at 2.5% Bov-Zn (Fig.1). FCS did not compete with Bov-Zn in suppressing TNF-α in LPS-stimulated monocytes. At low FCS concentrations Bov-Zn stimulated TNF-α production in the absence of LPS ($p<0.05$). This increase in TNF-α was countered with increasing concentrations of FCS (Fig.2, Treat x Dose: $p<0.01$). Cellular metabolism was not affected by 10% Bov-Zn (Fig. 3, Time x Treat: $F_{7,28} = 0.96$, n.s.; Treat: $F_{1,28} = 0.13$, n.s).

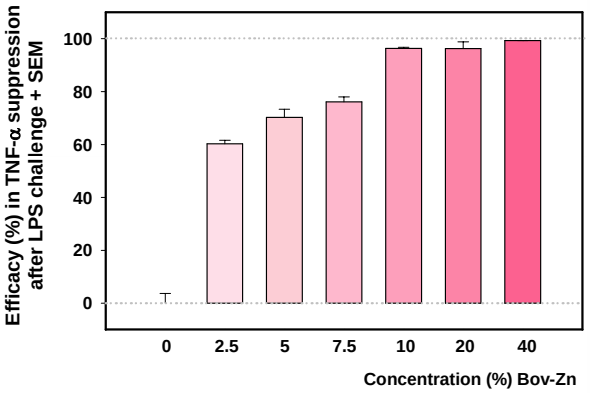

Fig. 1: Efficacy (%) of Bov-Zn in suppressing LPS-stimulated human monocyte TNF-α measured in supernatant after incubation with 0, 2.5, 5, 7.5, 10, 20, or 40% Bov-Zn for 24hrs.

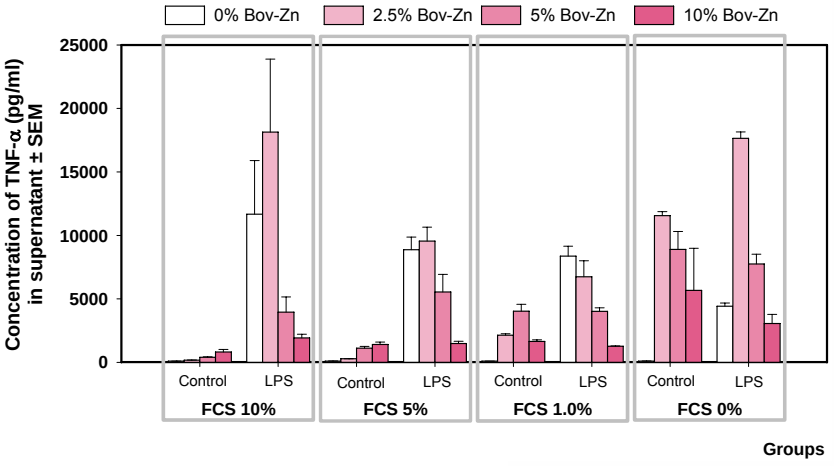

Fig. 2: Suppression of LPS-stimulated human monocyte TNF-α secretion in a competitive inhibition design for 0, 1, 5, 10% FCS against 0, 2.5, 5, 10% Bov-Zn.

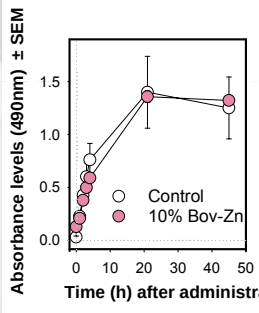

Fig. 3: Absorbance of formazan at 450nm, reflecting cell metabolism, of human monocytes incubated with 0% or 10% Bov-Zn. Triplicate samples were taken at 0, 1, 2, 3, 4, 5, 21 and 45hrs incubation.

CONCLUSION

Bov-Zn reduces LPS-stimulated human monocyte TNF-α secretion *in vitro*, without competing with FCS in the culture medium, and without disturbing the metabolism of monocytes.