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Effects of the prostaglandin analogue misoprostol on inflammatory mediator release by human monocytes

D.L. Widomski, R.E. Walsh, D.A. Baron[2], M.I. Hidvegi[2], D.J. Fretland, P.W. Collins and T.S. Gaginella[1]

Gastrointestinal Diseases Research and Pathology[2], Searle Research and Development, Skokie, IL 60077, USA

Abstract

The effect of misoprostol (M) on IL-1β, TNF-α, and lipid mediator release (assessed by RIA) by adherent (assessed by electron microscopy) human monocytes were studied *in vitro*. Human monocytes stimulated with E. Coli-derived lipopolysaccharide showed an increase in both IL-1β and TNF-α release. Incubation of the monocytes with LPS and M (18 hrs.), resulted in a reduction of both IL-1β and TNF-α levels. Leukotriene B_4 levels did not increase in response to LPS or M. LPS also caused an increase in thromboxane (TXB_2). *M* decreased TXB_2 levels. 6-keto PGF1α (6KP). Incubation with LPS and M stimulated release. LPS caused an increase in PGE_2 levels. M (100 μM) caused an increase in PGE_2 levels, M (1 μM) had no effect on PGE_2. These data suggest a possible immunomodulatory role for misoprostol in inflammatory diseases.

Introduction

Monocytes produce a variety of proinflammatory mediators in response to LPS [1]. These mediators, such as IL-1β and tumor necrosis factor-α, may be involved in inflammatory bowel disease (ulcerative colitis and Crohn's disease). Inhibition of these mediators could be of therapeutic value in IBD. Misoprostol [(±)-methyl 11a, 16-hydroxy-16-methyl-9-oxoprost-13E-1-oate] is a PGE_1 analogue reported to have immunomodulatory activity. Therefore, we studied its effects on the production of inflammatory mediators by human monocytes.

Materials and methods

A mixed population of human monocytes and lymphocytes was obtained by a discontinuous *metrizamide* gradient method [2]. Cells were pooled and resuspended in an α-modified Eagle's medium (α-MEM, Gibco) supplemented with 20 m*M* 3-(N-morpholino) propanesulfonic acid, 13.3 m*M* $NaHCO_3$, 2 m*M* glutamine, 50 μM 2-mercaptoethanol, 70 µg of streptomycin sulfate per ml and 1% fetal calf serum. Sterile plastic 16 mm wells were preincubated with media for 30 minutes at 37 °C in a 5% CO_2 incubator. 2.5×10^6 cells were added in a volume of one ml to each well and incubated for one hour at 37 °C in a CO_2 incubator. Three washes with cold phosphate buffered saline removed loosely adherent cells. One ml of media was added to each well. Lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4 (Sigma) was added to a final concentration of 100 ng/ml together with the appropriate concentration of misoprostol. The cells (90% pure) were then incubated for 18 hours at 37 °C in a CO_2 incubator. After 18 hours the supernatant

[1] Author for correspondence.

Table 1
Effect of LPS and Misoprostol (Miso) on inflammatory mediator release by human monocytes.

Mediator	*n*	Control	LPS 100 ng/ml	MISO 1 μM	MISO 100 μM
IL-1β (fmol)	16–21	1.35±0.62	3.94±0.82***	3.19±0.48**	1.26±0.24***
TXB_2 (ng/ml)	16–24	1.146±0.18	3.55±0.86***	1.35±0.125***	0.135±0.134***
6KP (ng/ml)	16–24	0.035±0.005	0.139±0.01***	0.172±0.01*	0.277±0.01***
TNFα (pg/ml)	16–24	161.3±20	340±52.4*	131.5±17.2*	120.3±12.8***
PGE_2 (μg/ml)	16–23	0.24±0.145	8.58±4.11	5.885±1.83	1669.5±268.5***

Values are means ±SE * $p<0.05$, ** $p<0.01$, *** $p<0.01$.

were removed, centrifuged ($170 \times g$ for 15 min) and stored at −20 °C until assayed [3]. Interleukin-1β and tumor necrosis factor-α levels were determined by validated radioimmunoassays (Amersham & Genzyme, respectively). 6KP, PGE_2, LTB_4 and TXB_2 levels were also determined by validated radioimmunoassays (NEN). Statistical assessment was by analysis of variance for each comparison, with a factor for treatment and a factor for donor. P values less than 0.05 were considered significant.

Results

Incubation of human monocytes with LPS caused a significant increase in IL-1β ($p<0.001$), TXB_2 ($p<0.001$), and 6KP ($p<0.001$) levels (Table 1). Misoprostol (1 μM) reduced IL-1β and TXB_2 ($p<0.001$) production. Misoprostol (1 μM) caused a significant increase ($p<0.05$) in 6KP release. At a higher concentration (100 μM), misoprostol caused a significant decrease in IL-1β ($p<0.001$) and TXB_2 ($p<0.001$) release, but 6KP levels increased significantly ($p<0.001$). These effects of misoprostol were concentration related. LPS significantly ($p<0.05$) increased TNF-α release. Both 1 μM and 100 μM of misoprostol caused a significant ($p<0.05$ and $p<0.001$ respectively) reduction in TNF-α release. PGE_2 levels were increased by LPS, but not by misoprostol (1 μM). Misoprostol (100 μM) significantly ($p<0.01$) stimulated PGE_2 production. LTB_4 levels were unaffected by LPS as all values were below sensitivity (0.625 ng/ml) of the RIA (data not shown).

Discussion

Misoprostol inhibited release of IL-1β from human monocytes *in vitro*, in contrast to previously reported data using naturally occurring prostaglandins [4]. TNF-α and thromboxane release were also inhibited by incubation with misoprostol. This prostaglandin also stimulated 6KP, which is indicative of enhanced prostacyclin activity. Misoprostol also dramatically increased PGE_2 levels. PGE's are believed to have potent anti-inflammatory properties [5] and have been shown to reduce lymphokine production in activated macrophages [6]. Furthermore, misoprostol has improved renal function in cyclosporine-treated renal transplant recipients and reduced the frequency of acute transplant rejection [7]. These results suggest that misoprostol may have a potential beneficial immunomodulatory role in inflammatory diseases.

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