Probucol suppresses ICAM-1 expression in rat mesangial cells: Possible role of IL-1

Toshihiro Sugiura, Akira Wada, Toshiki Moriyama, Masaru Horio, Naohiko Ueda, Enyu Imai, and Masatsugu Hori

The First Department of Medicine, Osaka University School of Medicine, Osaka National Hospital, and Department of Clinical Laboratory Science, Osaka University School of Medicine, Osaka; and Nara Institute of Science and Technology, Nara, Japan

Probucol suppresses ICAM-1 expression in rat mesangial cells: Possible role of IL-1. Interleukin-1 (IL-1) participates in the progression of glomerulonephritis by up-regulating intercellular adhesion molecule-1 (ICAM-1) expression in experimental glomerulonephritis. Probucol, an anti-hyperlipidemic agent, ameliorates some types of glomerulonephritis regardless of serum cholesterol levels, and is also reported to inhibit IL-1 release from macrophages in atherosclerotic lesions. However, little is known about the mechanism of this favorable action on glomerular injury. We examined whether or not probucol inhibits ICAM-1 expression by suppression of IL-1 action in cultured rat mesangial cells. In this brief report, we review the actions of probucol on IL-1 secretion and discuss the possible mechanism by which probucol may suppress the glomerular injury.

A number of studies have demonstrated the beneficial effects of anti-hyperlipidemic therapy on the progression of renal injury. Inhibition of 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase, a lipid-lowering agent reported to exhibit the greatest reduction in low density lipoprotein (LDL) in nephrotic syndrome, ameliorates proteinuria and glomerulosclerosis in various experimental models [1–5]. Probucol, an anti-hyperlipidemic agent originally developed as an antioxidant, also attenuates proteinuria and glomerulosclerosis in puromycin aminonucleoside nephritis (PAN) rats. Recent studies have shown that probucol reduced the extent of renal injury, as evidenced by proteinuria and glomerular lesions, in cholesterol-fed ExHC rats and in passive Heymann nephritis [reviewed in 6]. Probucol ameliorates the renal injury in the absence of significant reduction in plasma cholesterol levels in these models, suggesting that the effect produced is not simply due to its hypolipidemic effects. However, little is known about the mechanism of probucol’s action on glomerular injury.

In the present brief report, we focus on the action of probucol on interleukin-1 (IL-1) secretion and discuss the possible mechanism by which probucol may suppress glomerular injury.

ROLE OF INTERLEUKIN-1 IN GLOMERULONEPHRITIS

Recent studies have shown that many cytokines, growth factors and vasoactive substances participate in the development of glomerular lesions in experimental and human glomerulonephritis. Among these cytokines, IL-1 has been known to contribute to the early stage of inflammation in glomerulonephritis. IL-1 has two types of actions on mesangial cells (Fig. 1). One important action is to promote cell proliferation. Although IL-1 alone does not promote mesangial cell proliferation, it synergistically increases proliferation induced by platelet-derived growth factor (PDGF) or basic fibroblast growth factor (bFGF) in mesangial cells. We have demonstrated that this synergistic action of IL-1 is mediated through secretion of group II phospholipase A₂ (PLA₂) [3]. The other important action of IL-1 is to provoke leukocyte infiltration, which is mediated, at least in part, by up-regulating intercellular adhesion molecule-1 (ICAM-1) expression on mesangial cells.

The interaction between ICAM-1, a member of immunoglobulin superfamily expressed on a variety of cells including mesangial cells, and its counter receptor (lymphocyte function-associated antigen-1; LFA-1) play a key role in glomerular leukocyte infiltration in experimental models. IL-1 is reported to induce ICAM-1 expression in many types of cells in vitro. Northern blot analysis of ICAM-1 mRNA showed that exogenous IL-1 rapidly up-regulated ICAM-1 mRNA expression, with its peak at four to six hours after stimulation in cultured
Fig. 1. Schematic of interleukin-1 (IL-1) action on mesangial cells. IL-1 from infiltrating macrophages (Mφ) acts on mesangial cells (MC). IL-1 also acts in an autocrine or paracrine manner. One important action of IL-1 is to promote cell proliferation. IL-1 synergistically increases proliferation induced by PDGF or bFGF through the action of secreted group II PLA2. The other important role is to provoke leukocyte infiltration by increasing ICAM-1 expression. Probucol may inhibit ICAM-1 expression by suppression of IL-1 in an autocrine fashion.

Fig. 2. Northern blot analysis of intercellular adhesion molecule-1 (ICAM-1) mRNA expression. (A) Total RNA was isolated from rat mesangial cells treated with 200 U/ml IL-1β. Northern blot analysis, in which 20 μg of total RNA was applied in each line, was performed using 32P-labeled ICAM-1 cDNA. EtBr is ethidium bromide staining of ribosomal RNA. (B) Total RNA was isolated from rat mesangial cells treated with 10 μg/ml lipopolysaccharide (LPS) either in the presence or absence of 10 μg/ml probucol at six hours after stimulation. Northern blot analysis was performed using 32P-labeled ICAM-1 cDNA. The radioactivity of the hybridized probe was measured with a computing densitometer. Abbreviations are: C, control; L, 10 μg/ml LPS; L + P, 10 μg/ml LPS with 10 μg/ml probucol. Results are mean ± se of three experiments. *P < 0.05 vs. control. (C) Rat mesangial cells were treated as in Fig. 2B, and culture medium was collected at six hours after stimulation. IL-1 activity was measured by lectin-primed thymocyte proliferation assay. Results are mean ± se of four experiments. *P < 0.05 vs. control; **P < 0.05 vs. LPS stimulation. (Fig. 2B and C were reproduced from [2] with permission from Nephrology.)

Probucol inhibits ICAM-1 expression on mesangial cells

We have recently reported that probucol inhibits ICAM-1 mRNA and ICAM-1 protein expression by suppression of IL-1 activity in cultured rat mesangial cells [2]. Lipopolysaccharide (LPS), a strong stimulant to release rat mesangial cells (Fig. 2A). Indeed, IL-1-provoked leukocyte adhesion on cultured mesangial cells was inhibited by anti-ICAM-1 antibody [4].

In rat antiglomerular basement membrane (GBM) glomerulonephritis, treatment with IL-1 receptor antagonist (IL-1ra) has been shown to reduce glomerular injury and leukocyte infiltration [5, 6]. This suppression of tissue injury proved to be associated with the reduction of ICAM-1 expression, suggesting that IL-1 participated in the pathogenesis of glomerulonephritis by up-regulating ICAM-1 expression in this model [7]. Of note, the expression of ICAM-1 has been observed in mesangial cells in this model, which suggests the involvement of mesangial cells in the early phase of inflammatory reactions in this model. Considering these data, it may be beneficial to inhibit autocrine IL-1 secretion from mesangial cells so as to reduce this aspect of the inflammatory processes in glomerulonephritis.
IL-1 from mesangial cells, was shown to induce ICAM-1 mRNA expression on mesangial cells (Fig. 2B). Quiescent mesangial cells were incubated with LPS either in the presence or absence of probucol for 24 hours. LPS increased ICAM-1 mRNA expression by fivefold and probucol notably reduced this induction. In addition, IL-1 receptor antagonist (IL-1ra) suppressed LPS-induced ICAM-1 mRNA expression on mesangial cells in a dose-dependent manner and 100 ng/ml IL-1ra completely inhibited ICAM-1 expression, indicating that the induction was elicited through the action of secreted IL-1 in an autocrine manner [2]. It is interesting that the changes of ICAM-1 observed at mRNA levels were also confirmed at protein levels within 24 hours after stimulation by immunoblotting.

Probucol has been known to reduce the progression of atherosclerotic lesions. Atherosclerotic lesion results from the accumulation of foam cells of monocyte/macrophage origin within the arterial intima. IL-1 from these cells plays an important role in the formation of atherosclerotic lesion. Since probucol is reported to inhibit IL-1 release from macrophages, probucol may ameliorate atherosclerosis both by inhibiting IL-1 release from macrophages and by inhibiting the formation of oxidized LDL, which itself enhances IL-1 release from macrophages [8–10].

In this context, we proposed that probucol might suppress inflammatory processes of glomerulonephritis by inhibiting IL-1 release from mesangial cells. To determine whether or not probucol would reduce the IL-1 secretion from mesangial cells and consequently suppress ICAM-1 expression, IL-1 activity in the culture medium was measured by thymocyte proliferation assay. As shown in Fig. 2C, LPS increased IL-1 activity in the culture medium and probucol inhibited this activity significantly. The changes of ICAM-1 mRNA expression was in parallel with that of IL-1 activity. Our finding indicates that ICAM-1 induction by IL-1 in mesangial cells may play a relevant role in the formation of glomerular injury.

MECHANISM OF PROBUCOL’S ACTION ON IL-1 SYNTHESIS AND/OR RELEASE

Although mesangial cells induce IL-1 activity in response to LPS stimulation that is attenuated by probucol, its mechanism is largely obscure. The effects of probucol on IL-1 induction has been studied intensively using monocytes and macrophages. LPS affects the post-translational release of IL-1 as well as by increasing IL-1 mRNA expression. The effects of probucol on IL-1 are controversial. Probucol inhibits IL-1 mRNA expression in some cells, whereas it has no effect in others.

We have recently reported that LPS and probucol have no effect on IL-1β mRNA expression, suggesting that LPS and probucol might regulate IL-1 activity at the post-translational level in mesangial cells [2]. In addition, IL-1β processing and secretion is stringent and complex. IL-1β is synthesized as a 31 to 34 kDa precursor (proIL-1β) and is processed to mature 17 kDa biologically active form by IL-1β-converting enzyme (ICE) [9]. Probucol may affect the processing of IL-1 activation. Interestingly, nitric oxide (NO) was shown to increase IL-1 activity in murine macrophages. NO increases the IL-1 activity as well as the amount of IL-1 protein as determined enzyme-linked immunosorbent assay, not by an alteration of IL-1 expression nor by modification of IL-1 precursor processing, but by a cyclic GMP-dependent mechanism [10]. Therefore, LPS may increase IL-1 activity through the actions of NO, which is a reactive free radical, and probucol, an antioxidant, may inhibit the release of IL-1 activity by blocking NO. Further studies are necessary to clarify the mechanism responsible for the regulation of IL-1 activity.

CONCLUSION

Interleukin-1 and ICAM-1 play a key role in the early phase of inflammatory reactions in glomerulonephritis. We demonstrated that probucol inhibited LPS-induced ICAM-1 expression in cultured rat mesangial cells, and that this inhibition was mediated by blocking the release of IL-1 activity from these cells. These results throw light on the therapeutic application of probucol to the suppression of the inflammatory process of glomerulonephritis.

Reprint requests to Toshihiro Sugiura, M.D., The First Department of Medicine, Osaka University School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: sugiura@medone.med.osaka-u.ac.jp

REFERENCES

7. NIKOLIC-PATerson DJ, LAM HY, HILL PA, VANNICE JL, ATKINS RC: Suppression of experimental glomerulonephritis by the...