HYPOTHESIS

Lysophosphatidic acid and mesangial cells: implications for renal diseases

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ABSTRACT

The last decade has witnessed a phenomenal increase in our understanding of the biological role of lysophosphatidic acid (LPA) and has led to an appreciation of this critical serum-derived growth factor released from platelets. We herein summarize recent observations that collectively support the hypothesis that LPA may play a key role in the pathogenesis of initiation and progression of proliferative glomerulonephritis. LPA synergistically stimulates mesangial cell proliferation in combination with platelet-derived growth factor in primary culture. The mechanism of co-mitogenesis is likely to be mediated by the prolonged activation of mitogen-activated protein kinase which is stimulated by platelet-derived growth factor and LPA through different mechanisms. LPA contracts cultured mesangial cells and has properties in common with other pressor molecules including mobilization of intracellular Ca\(^{2+}\) and promotion of Ca\(^{2+}\) entry through dihydropyridine-sensitive calcium channels. LPA receptor mRNA has been identified in isolated glomeruli dissected from renal biopsy samples of patients with IgA nephropathy. All of these facts have led us to postulate that LPA is produced within glomeruli and that LPA’s mitogenic as well as haemodynamic action contribute to the pathological process of mesangial proliferative glomerulonephritis. The possible production of LPA as an autocrine factor from mesangial cells themselves has also been discussed.

INTRODUCTION

Lysophosphatidic acid (LPA; 1-acyl-sn-glycero-3-phospho), the simplest of all glycerophospholipids, is a key intermediate in the early steps of phospholipid biosynthesis in eukaryotic as well as prokaryotic cells [1]. LPA can also be generated naturally through the phospholipase-mediated hydrolysis of pre-existing phospholipids in the plasma membrane and released after cell activation in cultured cells [2,3]. LPA is abundantly produced and released by thrombin-activated platelets, and is thus a normal constituent of serum in the range of approximately 2–20 \(\mu M\) [4]. LPA binds strongly to serum albumin. Recently, the putative main metabolic pathways as well as the enzymes responsible for LPA generation in platelets have been identified, demonstrating that phospholipase C and phospholipase A\(_2\) are involved in the process of LPA synthesis [5]. LPA can be also generated as an enzymic breakdown product of lysophosphatidylincholines by the action of lysophospholipase D [6].

To date, a wide variety of biological activities of LPA

Key words: growth factor, lysophospholipid, platelets.
Abbreviations: GN, glomerulonephritis; LPA, lysophosphatidic acid; MAPK, mitogen-activated protein kinase; PDGF, platelet-derived growth factor; PLA\(_2\), phospholipase A\(_2\); PTX, pertussis toxin.
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has been reported. For example, exogenous LPA can promote the proliferation of fibroblasts, smooth muscle cells or epithelial cells [1], suggesting that LPA plays a role in the wound-healing process. On the other hand, LPA inhibits the growth and alters the shape of neuroblastoma cells [7], suggesting the role of neuronal plasticity. Besides these activities, LPA elicits various actions, including induction of invasion of tumour cells [8] and inhibition of apoptosis [9]. These diverse biological actions of LPA are mediated by its own G-protein-coupled receptors. In contrast to these biological effects of LPA in vitro, very little is known about its effects and mechanisms of action in vivo. So far, LPA is reported to aggregate platelets and induce species-specific dual pressor/depressor activity for systemic blood pressure in vivo [10,11].

Several groups have succeeded in isolating cDNAs encoding functional LPA receptors. As for human LPA receptors, two subtypes, termed Edg2 and Edg4, have been identified to date [12,13]. The discovery of these cDNAs provides definitive evidence of LPA being an extracellular mediator.

Recently, increasing evidence has accrued delineating an important role for platelets and platelet metabolites in mediating inflammatory and thrombotic kidney disorders including IgA nephropathy, lupus nephritis, haemolytic uraemic syndrome and chronic transplant rejection [14]. Intraglomerular platelets or their degradation products have been demonstrated in the glomeruli of biopsy samples from patients with these diseases [15,16]. Platelet depletion has been shown to significantly reduce the glomerular hypercellularity in anti-Thy-1 glomerulonephritis (GN) [17] or mesangial proliferative nephritis induced by Habu snake venom [18]. Because LPA is a substance released from aggregated platelets [10], we were interested in characterizing LPA’s effect on the function of mesangial cells. In this article, we cite several lines of experimental evidence that indicate the importance of LPA in the pathogenesis of GN.

**CO-MITOGENIC ACTION OF LPA WITH PLATELET-DERIVED GROWTH FACTOR**

LPA has been demonstrated to synergistically stimulate mesangial cell proliferation in combination with platelet-derived growth factor (PDGF) [19]. We assessed the mitogenic action of LPA, PDGF and the combination of LPA and PDGF in primary cultured rat mesangial cells by measuring \[^{3}H\]thymidine incorporation 24 h after the addition of these agonists under serum-free conditions. We also compared mesangial cell growth by counting the number of cells in LPA-, PDGF- or LPA/PDGF-treated dishes for 7 days. We demonstrated that although LPA itself was only weakly mitogenic for mesangial cells, LPA acted with PDGF to synergistically stimulate PDGF-induced DNA synthesis. Under maximal conditions obtained by the concentration of 30 μM LPA and 40 ng/ml PDGF, LPA/PDGF-induced \[^{3}H\]thymidine incorporation was found to be approximately 3.6 times higher than PDGF-induced incorporation. The action of LPA as a co-mitogen with PDGF was further confirmed by comparing cell growth. When cells were stimulated with both LPA (30 μM) and PDGF (40 ng/ml), the mean population doubling time was approximately 4.6 days. In contrast, the mean population doubling time of PDGF-treated dishes was approximately 8.6 days, which was significantly longer than that of LPA/PDGF-treated cells. When cells were treated with LPA alone, a significant increase in cell number compared with quiescent cells was not observed. Based on these experiments we concluded that, in contrast to its weak action as a mitogen by itself, LPA acts as a potent co-mitogen for mesangial cells, potentiating or magnifying the mitogenic effect of PDGF. It should be noted that this combined action of LPA with PDGF was nearly equivalent to that attained under ‘maximal culture conditions’, i.e. with media supplemented with 20% fetal bovine serum.

The precise mechanisms of the synergistic mitogenic action of PDGF and LPA have not yet been fully elucidated. However, further detailed analyses revealed that LPA and PDGF acted through different mechanisms to activate mitogen-activated protein kinase (MAPK) [20], a key element in cell proliferation, and that highly sustained activation of MAPK was achieved by combined stimulation with LPA and PDGF compared with stimulation by LPA or PDGF alone. For example, the MAPK activity induced by LPA, which peaked at 25 min after the stimulation, was inhibited by 41% pertussis toxin (PTX). In contrast, the peak activity of MAPK stimulated by PDGF at 10 min was not inhibited by PTX. Such a difference in the sensitivity to PTX was also observed in the mitogenic studies using LPA and PDGF: LPA-induced DNA synthesis was partially (approximately 31%) inhibited by PTX, whereas PDGF-induced mitogenic activity was not inhibited by PTX. On the other hand, both the synergistically stimulated DNA synthesis and the peak MAPK activity induced by the combination of LPA and PDGF were inhibited by PTX (by 18% and 21% respectively). These findings indicate that LPA and PDGF act by different mechanisms to activate MAPK and thus stimulate mesangial DNA synthesis. Furthermore, when cells were stimulated with both LPA and PDGF, the peak MAPK activity exceeded that induced by either LPA or PDGF alone, and the elevated activity was sustained for up to 50 min. Since published experimental data have demonstrated that MAPK acts as a switch kinase which can integrate signals from tyrosine kinase receptor and G-protein-coupled receptor [21], it is assumed that distinct signalling pathways triggered by LPA or PDGF in mesangial cells converge at the point of MAPK, leading to the sustained elevation of MAPK.
Figure 1 Model of the proliferation of mesangial cells through activation of MAPK

A schematic presentation of the regulation of MAPK by a tyrosine-kinase-coupled receptor (PDGF) and G-protein-coupled receptor (LPA). PDGF- and LPA-induced signalling pathways are distinct but interconnected at the point of MAPK to promote synergistic growth stimulation in rat mesangial cells.

levels as well as to a synergistic stimulation of cell growth (Figure 1).

**VASOPRESSOR ACTIVITY OF LPA**

LPA has been also demonstrated to act as a constrictor of rat mesangial cells. Exogenous LPA induces a contractile response in mesangial cells cultured on collagen gel, as assessed by a decrease in cell surface area. LPA was also found to induce mobilization of intracellular Ca\(^{2+}\) by activation of the phosphoinositide cascade as well as promotion of Ca\(^{2+}\) entry across the plasma membrane through dihydropyridine-sensitive calcium channels [22]. Calcium mobilization from intracellular stores and subsequent Ca\(^{2+}\) entry from the extracellular space into the intracellular cytoplasm is commonly observed in contractile cells stimulated by vasopressor substances [23,24]. In light of the demonstration that LPA-mediated mesangial contraction was completely inhibited by the calcium channel blocker isradipine [25], these observations suggest that LPA is a pressor molecule. Indeed, intravenous injection of LPA has been reported to induce systemic hypertension in experimental animals including rats [11]. Vasopressor molecules are suggested to reduce renal blood flow as well as glomerular filtration rate, thus contributing to the exacerbation of renal functions through their haemodynamic effects. Indeed, many kinds of vasopressor molecules which induce mesangial cell contraction in vitro, including angiotensin II, platelet-activating factor, thromboxane A\(_2\), histamine, epidermal growth factor and PDGF, have all been demonstrated to participate in the process of exacerbation or progression of diverse glomerular diseases [14,26,27]. In addition, it has been suggested that vasopressor molecules enhance the glomerular inflammatory process by inducing chemotaxis or promoting leucocyte adhesion [28]. Taking these findings together, when we consider that LPA shares a common property with other pressors, it is reasonable to assume that LPA may influence the pathogenesis of GN through its haemodynamic effects as well as by promoting the inflammatory process.

**HYPOTHETICAL MECHANISMS OF GN BY PDGF AND LPA**

Over the past few years, important insights into the role of PDGF in mediating renal disease progression have been made by the following lines of experimental evidence. Firstly, both PDGF and the PDGF receptor have been shown to be elevated in proliferating mesangial cells in vivo and to correlate with their hypercellularity [29,30]. Secondly, anti-PDGF IgG has been demonstrated to block mesangial cell proliferation in Thy-1 GN [31]. Thirdly, PDGF has been demonstrated to be released from tissue macrophages or activated monocytes [32]. Finally, the release of PDGF from mesangial cells has also been demonstrated by diverse stimuli including epidermal growth factor, tumour necrosis factor-\(\alpha\), basic fibroblast growth factor, endothelin-1 and thrombin [33,34], suggesting that PDGF is a growth factor that can act by itself and as an intermediary for other growth factors to stimulate mesangial cell proliferation. Furthermore, Terada et al. [35] have recently demonstrated the increased expression of PDGF-B-chain and PDGF receptor mRNA in kidney biopsy samples obtained from patients with severely advanced IgA nephropathy. Together, these data constitute a theoretical framework for postulating that PDGF is a key element in inducing a mesangial mitogenic response by autocrine or paracrine means, thereby mediating renal disease progression.

On the other hand, we do not yet know if LPA truly acts as an in vivo mitogen for glomerular mesangial cells. However, it has recently been suggested that LPA is generated and released as a product of arachidonic acid when group II phospholipase A\(_2\) (PLA\(_2\)) is activated in mesangial cells [36]. Group II PLA\(_2\) has been recognized as a mediator of inflammation in diverse diseases including GN and has been shown to be secreted from cultured mesangial cells in response to interleukin-1 stimulation [36]. Conversely, LPA was found to stimulate arachidonic acid metabolism, possibly through the activation of PLA\(_2\) [22]. In addition, significantly increased amounts of group II PLA\(_2\) were detected in human urine from patients with mesangial proliferative GN [37]. Therefore, it is reasonable to speculate that LPA may act as an autocrine factor in mediating the
inflammatory response of mesangial cells in vivo (Figure 2).

We now speculate as to how PDGF and LPA may interact and affect diseased mesangial cells. First, PDGF is released into the capillary lumen when platelets are activated or aggregated. At the same time, LPA is also released from aggregating platelets. These substances reach the mesangial cell surface from the capillary lumen through the fenestra of endothelial cells. When stimulated with PDGF, mesangial cells begin to produce and release PDGF, while the expression of PDGF receptor on the cell surface may be increased, leading to PDGF-dependent mesangial cell proliferation in an autocrine and paracrine manner. On the other hand, in diseased mesangial cells, group II PLA_2 may be produced and released from the cells, thus promoting the production and release of LPA from the cells. Given that LPA is also released from platelets, LPA is assumed to act as a paracrine and autocrine growth factor for mesangial cells. Finally, PDGF and LPA may co-operate to stimulate synergistic mesangial cell proliferation.

Using ^111^In-labelled platelets, Floege et al. [38] showed that glomerular platelet accumulation occurred concomitantly with the onset of mesangial cell proliferation. This observation may be relevant to our hypothesis in light of the demonstration that LPA is a potent inducer of platelet aggregation [10] as well as a substance released from activated platelets [5]. These observations are consistent with the postulate that LPA may enhance the initial aggregation response of platelets, acting in the positive feedback process of platelet aggregation (Figure 2).

**FUTURE PERSPECTIVES**

Since the establishment of the mesangial cell culture system in 1983 [39], it has not only provided unique opportunities to investigate the physiology of the renal mesangium, but has also led to enormous insights into the pathophysiology of diseased mesangial cells. Hitherto, the data currently available under standard culture conditions of mesangial cells in vitro have all exhibited the phenotype of pathologically activated cells in vivo, and there are no examples of synthetic activities or response patterns in vitro that do not have any in vivo correlates [40]. Here, we have summarized the experimental data obtained in in vitro studies using cultured mesangial cells, demonstrating that LPA acts synergistically with PDGF to stimulate mesangial cell growth, and have raised the possibility of a significant role of LPA in conjunction with PDGF at sites of injury and inflammation of mesangial proliferative GN. Given that these two factors are originally produced and released from activated platelets, it seems more than likely that they co-operate with each other.

Furthermore, we have recently detected LPA receptor (Edg4) mRNA in renal glomeruli isolated from biopsy...
samples of patients with mesangial proliferative GN (O. Hotta, Y. Kondo and C. N. Inoue, unpublished work). This shows that LPA is generated by and involved in the pathophysiological process of human GN. LPA has just emerged as a functional molecule for mesangial cells. Therefore, numerous questions remain. How is LPA produced within glomeruli? Which types of cells express LPA receptor(s) within glomeruli? Do LPA receptor(s) and PDGF receptor co-localize on mesangial cells?

In conclusion, we believe our hypothesis will provide an attractive platform for initiating future investigations to characterize the action of LPA in glomerular disorders. Such initiatives may produce a potentially novel and fruitful approach to further elucidation of the mechanisms underlying the initiation and progression of GN.

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