Invited Comment

Angiotensin II and oxidized LDL: an unholy alliance creating oxidative stress

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General pathophysiological consequences of enhanced oxidative stress for vascular and renal function

Reactive oxygen species are produced continuously in many, not only vascular or renal, tissues and are part of the unspecific defense system. However, in various vascular or renal diseases enhanced formation of reactive oxygen species is thought to be pathogenic, e.g. in atherosclerosis, glomerular disease, renal failure, pyelonephritis or aminoglycoside nephropathy [1–4]. In the vascular system, the formation of O$_2^\cdot$ from endothelial cells, smooth muscle cells and macrophages seems to be of major relevance [3] mainly due to its reaction with nitric oxide (NO), particularly in the setting of hypercholesterolemia and atherosclerosis [5]. NO, the most important endothelium-derived relaxing factor, is scavenged by O$_2^\cdot$ [6,7]. NO reacts with O$_2^\cdot$ to yield peroxynitrite (ONOO$^-$), which is rather stable but can rearrange to form nitrate and the highly reactive OH$^\cdot$ radical. Important pathophysiological consequences of enhanced oxygen radical formation for the vascular system are: (i) attenuation of the endothelium-dependent dilation, resulting in disturbed organ perfusion and systemic hypertension [8]; (ii) production of OH$^\cdot$ radicals, which cause cellular damage and may contribute to inflammation [9]; and (iii) induction of apoptosis [10,11]. In the kidneys, O$_2^\cdot$ production has been detected in vascular cells, juxtaglomerular cells, tubular cells, podocytes, mesangial cells and isolated glomeruli. One example of a renal disease where enhanced formation of reactive oxygen species may play a causal role is membranous nephropathy [12]. In passive Heymann nephritis (PHN), an experimental form of membranous nephropathy, it was demonstrated that H$_2$O$_2$ is formed and leads to glomerular damage [13]. Other important consequences of enhanced oxidative stress for renal function are stimulation of renin release from juxtaglomerular cells, glomerular injury, stimulation of mesangial cell growth and hypertrophy of tubular epithelial cells [4,14–16].

Specific effects of Ang II and OxLDL

Evidence for enhanced oxidative stress induced by Ang II

Cell culture studies with rat smooth muscle cell preparations provided the first experimental evidence for stimulation of O$_2^\cdot$ production by Ang II [17]. In a more recent investigation, a constitutive O$_2^\cdot$ generating activity was localized in the rabbit aortic adventitia [18]. This constitutive O$_2^\cdot$ production could be enhanced by Ang II, confirming the first study. In the kidney, Ang II stimulates O$_2^\cdot$ production in cultured LLC-PK1, mouse tubule cells [14] and mesangial cells [16]. In addition, increased peroxide formation in macrophages was demonstrated after stimulation with Ang II via the AT$_1$ receptor [19].

Mechanism of Ang II-induced O$_2^\cdot$ production

In experiments with rat smooth muscle cell membranes, Ang II-induced stimulation of O$_2^\cdot$ production could be inhibited by diphenylene iodonium, suggesting that O$_2^\cdot$ was produced by membrane-bound NADH/NADPH oxidases [17]. The involvement of the NADH/NADPH oxidases was also detected in rabbit aortic adventitia preparations [18], in cultured mesangial cells [16] and in mouse tubule cells [14]. The Ang II stimulation was transmitted via the AT$_1$ receptor [14,16,19], and involved the NADPH subunits p22(phox) [the alpha subunit of cytochrome b(558) and part of the electron transfer component of the phagocytic NADPH oxidase] and p67(phox).

Functional consequence of Ang II-induced oxidative stress

General pathophysiological consequences of enhanced oxidative stress have been outlined briefly above. The particular importance of Ang II-induced oxidative
stress has been investigated in the context of vasomotor tone and cell-cycle regulation. In a rat model of Ang II-induced hypertension, it has been shown that vascular \( \text{O}_2^- \) production was enhanced after Ang II treatment, but not after treatment with the control vasoconstrictor norepinephrine [20]. Enhanced \( \text{O}_2^- \) production resulted in impairment of endothelium-dependent dilation [21]. This could be prevented by liposome-encapsulated superoxide dismutase (SOD). The AT\(_1\)-receptor antagonist losartan also prevented impairment of endothelium-dependent dilation, demonstrating that the AT\(_1\) receptor was involved. In the rat two kidney–one clip model of hypertension, similar results were obtained [22]. In that study, endothelial cell dysfunction was improved by pre-incubation of vascular tissue with SOD and calphostin C, indicating that increased vascular \( \text{O}_2^- \) production was secondary to a protein kinase C (PKC)-mediated activation of a membrane-associated NAD(P)H-dependent oxidase.

Indirect evidence that Ang II-induced \( \text{O}_2^- \) production takes place in vivo in humans was provided by a study using forearm plethysmography, which allows direct measurement of Ang II-induced vasomotor actions. Constrictor actions of Ang II in the human forearm were enhanced during NO inhibition and were attenuated during vitamin C infusion, suggesting Ang II-associated stimulation of endothelial NO and of oxygen radicals, respectively [23].

Ang II induces \( \text{O}_2^- \) production in cultured LLC-PK1 and induces p27(Kip1) expression and hypertrophy in mouse proximal tubular epithelial cells. These findings suggest a novel mechanism of cell cycle modulation by Ang II [14]. In cultured rat mesangial cells, Ang II induced \( \text{O}_2^- \) production. In parallel, it increased \([^{3}H]\)-leucine incorporation and mesangial cell protein content, two markers of cellular hypertrophy, as well as \([^{3}H]\)-thymidine incorporation, a marker of hyperplasia [16]. Further evidence for a role of Ang II-induced \( \text{O}_2^- \) production in the genesis of cell hypertrophy comes from experiments with cultured vascular smooth muscle cells: inhibition of p22(phox) mRNA expression in vascular smooth muscle cells interfered with Ang II-stimulated processes, i.e. (i) \( \text{NADH/NADPH} \)-dependent superoxide production, (ii) subsequent hydrogen peroxide production, and (iii) \([^{3}H]\)-leucine incorporation [24].

Thus, Ang II-induced \( \text{O}_2^- \) production has important consequences for the regulation of vascular tone and cell cycle.

Evidence for enhanced oxidative stress induced by OxLDL

Animal studies with cholesterol-fed rabbits provided indirect evidence for a role of low-density lipoprotein (LDL) in the induction of oxidative stress. Aortas from hypercholesterolemic rabbits produced significantly more superoxide than control aortas [25,26]. Later, our group provided direct evidence that incubation of cultured human umbilical vein endothelial cells (HUVEC) and of isolated arteries with oxidized LDL or lipoprotein(a) [Lp(a)] stimulated \( \text{O}_2^- \) production (Fig. 1) [10,15,27,28]. Induction of free radical formation has also been demonstrated in Lp(a)-stimulated macrophages [29] and in OxLDL-stimulated mesangial cells [30].

**Mechanism of OxLDL-induced \( \text{O}_2^- \) production**

The mechanism through which \( \text{O}_2^- \) production is stimulated has still to be elucidated in detail. Generally, oxidation of LDL is a prerequisite for the ability of LDL to stimulate \( \text{O}_2^- \) production. This observation points to a role of products of the lipid peroxidation process. There is also evidence that specific receptors for OxLDL are involved which are distinct from the classic ApoB100 receptor for native LDL. During lipoprotein oxidation, various products are formed which are more or less stable, including lysophosphatidylcholine, aldehyde lipid peroxidation products, and fatty acids produced by phospholipase A\(_2\) [31–33]. Lysophosphatidylcholine, a by-product of cholesterol esterification, increases \( \text{O}_2^- \) production in vascular smooth muscle cells via stimulation of PKC [34]. A similar mechanism may take place in endothelial cells and other cells. At present, information on the role of the specific OxLDL receptors in the production of oxygen radicals is not available, e.g. the endothelial LOX-1.

**Functional consequence of OxLDL-induced oxidative stress**

There is a vast literature on effects of OxLDL in the context of atherosclerosis, vasomotor regulation and endothelial function which is beyond the scope of this text. Here we discuss only those effects of OxLDL that have been linked directly to enhanced oxidative stress. These comprise the impact of OxLDL-induced production of oxygen radicals on: (i) regulation of vascular tone, (ii) renin release and (iii) apoptotic cell death. \( \text{O}_2^- \) interacts with NO, resulting in its inactivation [6]. Through inactivation of NO, OxLDL affects endothelial function and impairs endothelium-dependent dilation [27,28,35]. The latter is an important factor in the genesis of disturbed organ perfusion, e.g. in the coronary circulation [35]. The impact of OxLDL on apoptotic cell death provides another potentially important pathway in the development of atherosclerosis and glomerulosclerosis. OxLDL induces apoptosis in cultured HUVEC (Fig. 2) [10,11,36] and in smooth muscle cells of isolated aorta [10]. Furthermore, OxLDL induces apoptosis in cultured mouse mesangial cells [30]. In all the cited studies the use of antioxidants (SOD, vitamin C/E, or butylated hydroxytoluene) prevented apoptosis. Another renal effect was documented in isolated juxtaglomerular cells in primary culture: OxLDL stimulated renin release and this effect was linked to stimulation of \( \text{O}_2^- \) production [15,37].
Angiotensin II and oxidized LDL

Fig 1. OxLDL and OxLp(a) stimulate O$_2^-$ production in cultured endothelial cells.

Co-localization of OxLDL and Ang II

Accumulation of OxLDL in atherosclerotic plaques and in the glomerulus is well known and may play an important role in the development of atherosclerosis and glomerulosclerosis [44–47]. As shown recently, the concentration of Ang II is increased in atherosclerotic arteries, e.g. in human atherectomy preparations and the arteries of hypercholesterolaemic monkeys. Ang II co-localizes with OxLDL in resident macrophages [38].

Clinical studies

Animal studies indicated that ACE inhibition is beneficial for endothelial function in hypercholesterolaemia [48]. Two multicentre, prospective, controlled studies investigated the effect of the ACE inhibitor quinaprilat on endothelial function in humans (TREND = trial on reversal of endothelial dysfunction and QUIET = quinapril ischemic event trial [39,49]). Subgroup analysis in both studies revealed that the beneficial effect of ACE inhibition on endothelial cell function was particularly pronounced in patients with high serum LDL cholesterol (> 125 mg/dl). This observation is consistent with the idea that the superoxide-generating effects...
of Ang II have been attenuated interfering with the production of OxLDL.

Receptor expression

Recently several studies showed that the expression of the two receptors, i.e. the OxLDL receptor LOX-1 and the AT1 receptor, is stimulated by the respective other receptor agonist [41–43]. In cultured smooth muscle cells, LDL induced expression of the AT1 receptor [41]. In line with this finding, higher expression of the AT1 receptor on the surface of the aorta was found in hypercholesterolaemic compared with normcholesterol-olaemic rabbits [42]. Thus, LDL may sensitize the vascular tissue to the action of Ang II. However, expression of the OxLDL receptor LOX-1 and uptake of OxLDL in HUVEC is stimulated by Ang II [43].

Conclusion

There is no doubt that Ang II and OxLDL are potent agonists with effects on vascular and renal function. There is increasing evidence that many of their effects are mediated via enhanced oxidative stress. Recent experimental and clinical findings point to interaction between Ang II and OxLDL. This may be pathophysio- logically important and provide new options for therapeutic strategies.

Acknowledgements. This study was supported by grants from the Deutsche Forschungsgemeinschaft to J. G. (Ga 431/2-2).

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Editor’s note

Please see also the Original Article by Lim et al. (pp. 2680–2687 in this issue).