Nephrol Dial Transplant (2002) 17 [Suppl 9]: 84–87

Nephrology
Dialysis
Transplantation

Intervention by retinoic acid in oxidative stress-induced apoptosis

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Abstract
Retinoic acid (RA) has been considered a pro-apoptotic agent, and little is known about its anti-apoptotic potential. In this article, we describe that RA strongly inhibits hydrogen peroxide (H_2O_2)-induced apoptosis of mesangial cells by intervention in activator protein 1 (AP-1). Our data showed that: (i) H_2O_2 induces apoptosis of mesangial cells via the AP-1 pathway; (iii) activation of AP-1 by H_2O_2 is mediated by the c-Jun N-terminal kinase (JNK)–c-Jun/AP-1 pathway and the extracellular signal-regulated kinase–c-Fos/AP-1 pathway; (iii) RA inhibits H_2O_2-induced apoptosis via suppression of c-*fos*/c-*jun* expression and JNK activation; and (iv) the anti-apoptotic effect of RA is, at least in part, mediated by induction of mitogen-activated protein kinase phosphatase 1.

Keywords: activator protein 1; apoptosis; MAP kinase phosphatase 1 (MKP-1); mitogen-activated protein (MAP) kinase; oxidative stress; retinoic acid

Introduction

Apoptosis of glomerular cells is observed during the process of glomerulonephritis. The molecular mechanisms involved in the *in vivo* induction of apoptosis have not been identified yet, but several possibilities have been postulated. During initiation and progression of inflammation, toxic substances elaborated by leukocytes may induce apoptosis of glomerular cells. Putative triggers include tumour necrosis factor-α (TNF-α), Fas ligand and reactive oxygen intermediates [1]. In mesangial cells, reactive oxygen species including superoxide anion, hydrogen peroxide (H_2O_2) and peroxynitrite trigger apoptosis *in vitro* [2,3]. For example, mesangial cells exposed to H_2O_2 exhibit (i) shrinkage of cytoplasm, membrane blebbing and condensation of nuclei; (ii) DNA ladder formation; and (iii) positiveness for terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) [4].

Role of the activator protein 1 (AP-1) pathway in H_2O_2-induced apoptosis of mesangial cells

Multiple signalling pathways may be involved in oxidant stress-induced apoptosis of glomerular cells. The AP-1 pathway is a possible candidate. AP-1 is regarded as a redox-sensitive transcription factor, and several reports showed the importance of c-Jun N-terminal kinase (JNK) and its substrate c-Jun in the signalling pathways to apoptosis triggered by UV light, γ-irradiation, TNF-α and ceramide.

We previously reported that H_2O_2 induced expression of c-*fos* and c-*jun* and activation of AP-1 in cultured mesangial cells [5,6]. Down-regulation of AP-1 using either a dominant-negative mutant of c-Jun, an antisense c-*jun* or a pharmacological inhibitor of c-Jun/AP-1 attenuated the H_2O_2-initiated apoptosis [5]. These data suggested that H_2O_2 induces apoptosis of mesangial cells via the AP-1 pathway. In contrast, inhibition of another redox-sensitive transcription factor, nuclear factor-κB (NF-κB), via either a dominant-negative mutant of p50 NF-κB subunit or a super-repressor mutant of IκBα did not affect the H_2O_2-induced apoptosis [1,5].

Roles of the mitogen-activated protein (MAP) kinase family in the activation of AP-1 in H_2O_2-exposed mesangial cells

The transacting potential of AP-1 depends on its induction and phosphorylation by the MAP kinase

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family of molecules [7]. For example, expression of c-*fos* is regulated by ternary complex factors whose activity is regulated by extracellular signal-regulated kinase (ERK), p38 MAP kinase and JNK. Expression of c-*jun* is regulated by c-Jun and ATF-2 that are phosphorylated by JNK and/or p38 MAP kinase. Post-translational activation of AP-1 is also regulated by MAP kinase-mediated phosphorylation, i.e. c-Jun is phosphorylated and activated by JNK, and c-Fos is phosphorylated by a member of the MAP kinase family, Fos-regulating kinase [7]. MAP kinases possibly are involved in the induction of apoptosis by H_2O_2.

We found that mesangial cells exposed to H_2O_2 exhibited rapid phosphorylation of JNK, ERK and p38 MAP kinase [8]. Inhibition of ERK or JNK by pharmacological inhibitors (PD098059 and curcumin) attenuated H_2O_2-induced apoptosis. In contrast, the p38 MAP kinase inhibitor SB203580 did not improve cell survival. Consistently, transfection with dominant-negative mutants of ERK1 and ERK2 or a dominant-negative mutant of JNK inhibited H_2O_2-induced apoptosis. Transfection with a dominant-negative p38 MAP kinase did not attenuate the apoptotic process [8]. Inhibition of ERK by PD098059 suppressed induction of c-*fos* without affecting early induction of c-*jun*, leading to attenuated activation of AP-1 in response to H_2O_2 [8]. These results suggested that (i) activation of JNK and ERK, but not p38 kinase, is required for the H_2O_2-induced apoptosis; and (ii) the JNK–c-Jun/AP-1 pathway and the ERK–c-Fos/AP-1 pathway are involved in the induction of apoptosis by H_2O_2.

Intervention by retinoic acid (RA) in H_2O_2-induced apoptosis via inhibition of the JNK–AP-1 pathway

RA is an active metabolite of vitamin A and regulates a wide range of biological processes including cell proliferation, differentiation and morphogenesis [9]. The action of retinoids, including RA, is mediated by specific nuclear receptors; retinoic acid receptors (RAR-α, -β, -γ) and retinoid X receptors (RXR-α, -β, -γ). RXRs form homodimers and heterodimers with RARs or other nuclear hormone receptors and function as transcriptional regulators [10]. Previous reports showed that RA is a potent inhibitor of AP-1 [11]. If so, RA may inhibit H_2O_2-induced apoptosis.

In general, RA is known to induce apoptosis in various cell types including tumour cells and embryonic cells. In contrast, little is known about its anti-apoptotic potential. We examined effects of all-*trans*-RA (t-RA) and 9-*cis*-RA on H_2O_2-induced apoptosis of mesangial cells. Hoechst staining showed that pre-treatment with either agent suppressed the apoptotic process in a dose-dependent manner. It was associated with inhibition of c-*fos*/c-*jun* expression, suppression of JNK activation and attenuated AP-1 activity [6]. These data suggested the novel potential of RA as an inhibitor of apoptosis. The anti-apoptotic action of t-RA was ascribed, at least in part, to suppression of the cell death pathway mediated by JNK–c-Jun/AP-1.

We further investigated involvement of RAR and RXR in the anti-apoptotic effect of RA [12]. We found that pre-treatment with an RAR pan-antagonist or an RXR pan-antagonist attenuated the anti-apoptotic effect of t-RA. Similarly, transient transfection with a dominant-negative mutant of RAR or of RXR diminished the anti-apoptotic effect of t-RA. Both RAR and RXR antagonists reversed the suppressive effect of t-RA on AP-1 activity. However, the roles of RAR and RXR in the suppression of AP-1 components by t-RA were found to be different. RAR antagonist reversed the suppressive effect of t-RA on both c-*fos* and c-*jun*, whereas RXR antagonist reversed only the effect of t-RA on c-*fos*, but not c-*jun*. Furthermore, suppression of JNK activation by t-RA was observed even in the presence of RAR and RXR antagonists. Consistently, suppression of JNK by t-RA was not affected by overexpression of either the dominant-negative RAR or RXR [12]. These data elucidated that the anti-apoptotic effect of RA is mediated by both nuclear receptor-dependent and -independent mechanisms.

Involvement of MAP kinase phosphatase 1 (MKP-1) in mediating the anti-apoptotic effect of RA

MAP kinases are activated by upstream kinases including MEK1 and 2, and MKK3, 4 and 6. Once activated, however, these MAP kinases are inactivated rapidly by the family of protein phosphatases. In particular, dual-specificity protein phosphatases play crucial roles in the dephosphorylation and inactivation of MAP kinases. MKP-1, also termed CL100, 3CH134 and ERP, is a prototypic member of the family of inducible dual-specificity phosphatases [13]. It selectively dephosphorylates tyrosine and threonine residues on MAP kinases and inactivates them. To investigate further the anti-apoptotic effect of t-RA, we focused on a role for MKP-1. Northern blot analysis showed that, in mesangial cells, t-RA dose-dependently induced expression of MKP-1 at the transcriptional level [14]. The anti-apoptotic effect of t-RA on H_2O_2-induced apoptosis was observed in mesangial cells and NRK49F cells in which MKP-1 was inducible by t-RA. In contrast, the anti-apoptotic effect of t-RA was not observed in MDCK cells and ECV304 cells in which MKP-1 was not inducible. Inhibition of MKP-1 by vanadate, a protein tyrosine phosphatase inhibitor, attenuated the anti-apoptotic effect of t-RA. Furthermore, transfection with MKP-1 markedly inhibited H_2O_2-induced apoptosis of mesangial cells [14]. These data suggested that the

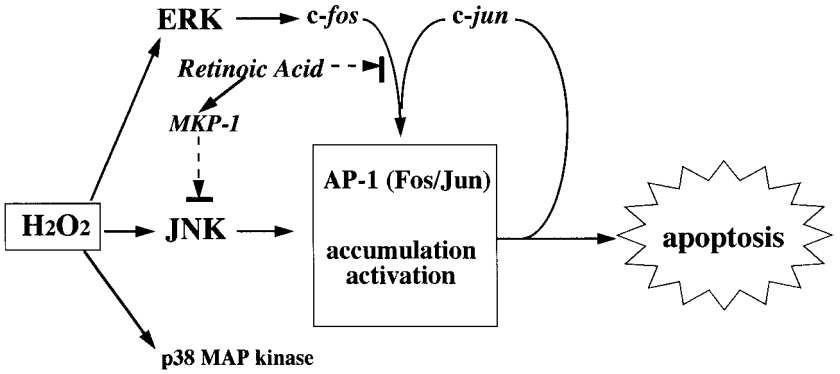

Fig. 1. Signal transduction pathways involved in hydrogen peroxide (H_2O_2)-induced apoptosis and intervention by retinoic acid. H_2O_2 induces apoptosis of mesangial cells via activation of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK). p38 mitogen-activated protein (MAP) kinase is also activated by H_2O_2, but it does not participate in the induction of apoptosis. ERK and JNK induce accumulation and activation of activator protein-1 (AP-1), leading to apoptosis. Retinoic acid inhibits the apoptotic process by suppression of AP-1, i.e. retinoic acid attenuates expression of c-*fos*/c-*jun* and activation of JNK, at least in part, via induction of the dual-specificity protein phosphatase, MAP kinase phosphatase 1 (MKP-1).

anti-apoptotic effect of t-RA was, at least in part, mediated by induction of MKP-1.

In summary, our data suggested that: (i) H_2O_2 induces apoptosis of mesangial cells via the AP-1 pathway; (ii) activation of AP-1 by H_2O_2 is mediated by the JNK–c-Jun/AP-1 pathway and the ERK–c-Fos/AP-1 pathway; (iii) RA inhibits H_2O_2-induced apoptosis via suppression of c-*fos*/c-*jun* expression and JNK activation; and (iv) the anti-apoptotic effect of RA is, at least in part, mediated by induction of MKP-1. The current hypothesis is summarized in Figure 1.

Perspective

In this article, we described the anti-apoptotic potential of RA in glomerular cells. The entire spectrum of pharmacological effects of RA on glomerular cells is largely unknown, but recent investigation suggested the utility of this agent for the treatment of glomerular diseases. *In vitro*, RA inhibits not only apoptosis but also mitosis of mesangial cells [15]. It also inhibits expression of several inflammatory mediators including inducible nitric oxide synthase, osteopontin and monocyte chemoattractant protein 1 [16–18]. *In vivo*, administration with RA dramatically suppresses development of anti-Thy 1 glomerulonephritis [19]. Further investigation of the biological effects of RA on glomerular cells would open a window towards therapeutic intervention in glomerular diseases.

Acknowledgements. This work was supported, in part, by grants from the Wellcome Trust and National Kidney Research Fund to M.K.

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