11 Transcription factors HSF and NF-κB as targets for cytoprotective eicosanoids: a new strategy for therapeutic intervention

M. G. SANTORO

The observation that an increase in temperature of a few degrees above the physiological level induces the synthesis of a small number of proteins in *Drosophila* salivary glands led to the discovery of a universal protective mechanism which prokaryotic and eukaryotic cells utilize to preserve cellular function and homeostasis[1]. This complex physiological defence mechanism, known as the heat shock response, involves the rapid induction of a specific set of genes encoding cytoprotective proteins (heat shock protein, HSP). In mammalian cells HSP synthesis is induced in a wide variety of toxic conditions, including extreme temperatures, oxidative stress, exposure to heavy metals or cytotoxic drugs, glucose deprivation and virus infection[1]. Whereas HSP induction was at first interpreted as a signal for detection of physiological stress, it is now well-documented that HSPs are utilized by the cells in the repair process following different types of injury, to prevent damage resulting from the accumulation and aggregation of non-native proteins[2].

Induction requires the activation and translocation to the nucleus of a transregulatory protein, the heat shock transcription factor (HSF). In mammalian cells, HSF exists as an inactive non-DNA binding form, which is rapidly converted to the DNA-binding form upon exposure to heat shock or other stimuli[3]. HSF activation is a multistep process and requires oligomerization, acquisition of DNA-binding activity, localization to the nucleus and phosphorylation by an as yet unknown kinase[3]. In the nucleus HSF binds to specific promoter elements (HSE) located upstream of heat shock (*hs*) genes, activating transcription. Several HSFs (HSF1–HSF4) have been identified in vertebrate cells [3]; however, the molecular mechanisms responsible for signal transduction which leads to HSF activation and phosphorylation have not been completely elucidated.

HSP can be divided into five families, depending on their molecular weight. In eukaryotic cells 70 kDa heat shock proteins (hsp 70), which are encoded by a large multigene family, include the constitutively expressed hsc70, the major inducible hsp70, the inducible hsp72, the glucose regulated grp78/BiP and the mitochondrial P75[3]. In mammalian cells, several HSP that function as molecular chaperones and are essential for a correct folding, assembly and intracellular translocation of proteins, are expressed

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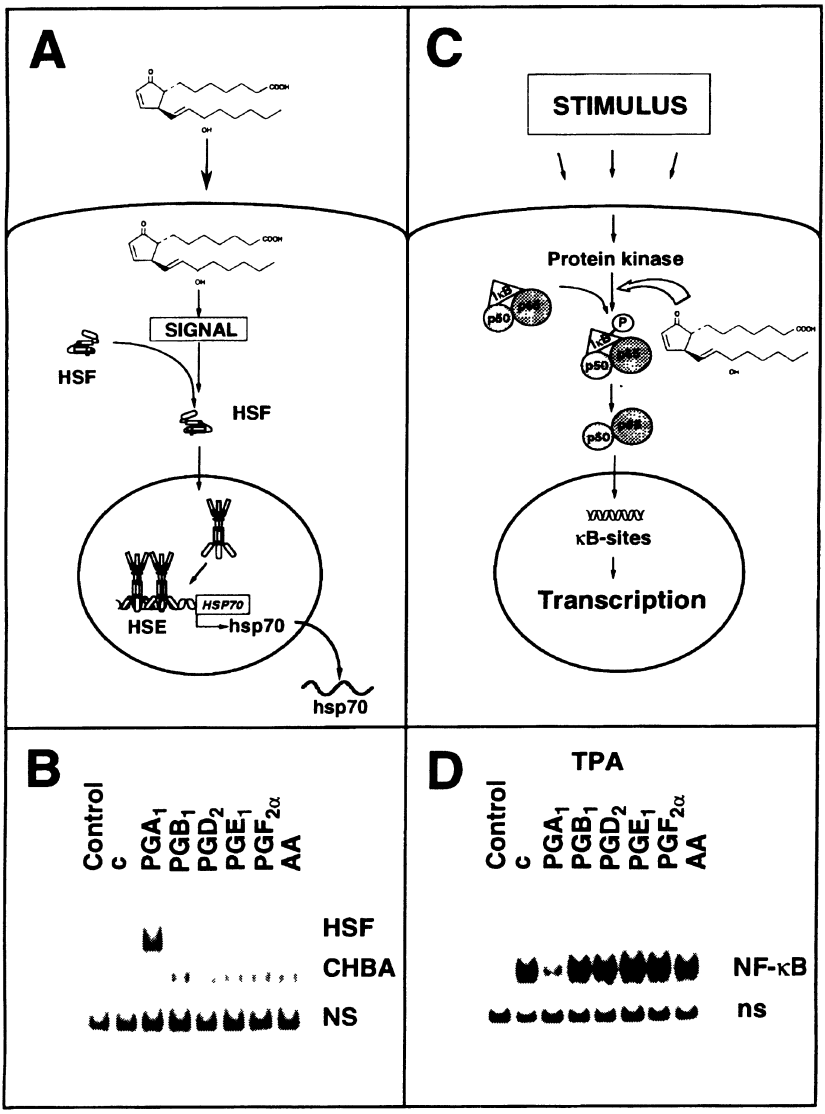
during normal growth conditions and can be induced by biologically active molecules such as haemin[4] and prostaglandins[5].

CYTOPROTECTIVE ROLE OF HEAT SHOCK PROTEINS IN HUMAN DISEASES

In the last few years, the cytoprotective role of HSP has been described in several human diseases, including metabolic disorders[6], inflammation[7], infection[8] and ischaemia[9]. In cardiac tissues a wide variety of insults, including myocardial ischaemia, trauma and hyperthermia, results in the synthesis of HSP, which have been shown to play a pivotal role in restoring normal cardiac function after injury, possibly by removal of denatured cardiac proteins and re-establishment of normal cardiac protein synthesis. A correlation has been shown between the amount of hsp70 and the degree of myocardial protection in vitro as well as in animal models[9,10]. Moreover, transfected heart-derived cells overexpressing hsp70 and hsp70-transgenic mice show enhanced resistance to ischaemic stress, providing evidence for a direct role of this protein in cytoprotection after this type of injury[9,11]. A pharmacological approach to hsp70 induction and cardiac protection is suggested by the recently described hsp70-induced protection by simulated ischaemia in rat neonatal cardiomyocytes treated with the hsp70 inducer herbimycin-A[12]. In the case of inflammation, HSP have been shown to protect mammalian cells from tumour necrosis factors α- and β-mediated cytotoxicity[13], as well as to suppress astroglial-inducible nitric oxide synthase expression[14]. Moreover, in a rodent model for adult respiratory distress syndrome (ARDS), pre-exposure of animals to heat shock, leading to massive induction of hsp70 within

Figure 1 Activation of the heat-shock response and inhibition of NF-κB by cyclopentenone prostaglandins. (A) Synthesis of cytoprotective heat-shock proteins is induced by PG via the activation of heat-shock transcription factor HSF. HSF converts from a monomeric non-DNA-binding form to an oligomeric form that translocates to the nucleus and binds to specific promoter elements (HSE) located upstream of heat-shock genes (e.g. HSP70). (B) Effect of arachidonic acid metabolites on HSF activation. Whole-cell extracts of Jurkat cells treated with 24 μM arachidonic acid (AA), PGA_1, PGB_1, PGD_2, PGE_1, $PGF_2\alpha$ or diluent control (c) for 6 h were incubated with a ^{32}P-labelled HSE probe, followed by analysis of DNA-binding activity by EMSA (21). Position of HSF-DNA binding complex (HSF), constitutive HSE-binding activity (CHBA) and non-specific protein –DNA interactions (NS) are shown. (C) Inhibition of NF-κB activation. NF-κB normally exists in an inactive cytoplasmic complex, whose predominant form is a heterodimer composed of p50 and p65 subunits, bound to the inhibitory protein IκBα. Cyclopentenone prostaglandins act by inhibiting IκBα phosphorylation and degradation. (D) Whole-cell extracts from Jurkat cells treated with AA, PGA_1, PGB_1, PGD_2, PGE_1, $PGF_2\alpha$ or diluent control (c) for 2 h and then stimulated with 12-O-tetradecanoylphorbol 13-acetate (TPA) for 6 h were incubated with a ^{32}P-labelled κB DNA probe, followed by EMSA. Position of NF-κB-DNA complex (NF-κB) and non-specific binding (ns) are indicated. Control cells are neither stimulated with TPA nor treated with prostaglandins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the lung, is associated with decreased pulmonary inflammation and prevention of death[15]. Increased expression of hsp70 has also been associated with inhibition of virus replication during acute infection[8,16]. These observations suggest new therapeutic strategies relying upon the development of drugs which selectively turn on heat shock genes.

EICOSANOIDS AND THE HEAT SHOCK RESPONSE

We have shown that cyclooxygenase cyclopentenone metabolites such as prostaglandins of the A and J type (PGAs and PGJs) induce the synthesis of hsp70 in a non-stressful situation in a wide variety of human and mammalian cells (reviewed in 17,18), whereas leukotrienes, and in particular LTB_4, do not activate HSF nor stimulate HSP expression in human and murine cells (Elia G, Santoro MG, unpublished results). The mechanism of hsp70 induction by cyclopentenone prostaglandins via cycloheximide-sensitive activation of HSF1 (Figure 1) and the kinetics of *hsp70* gene transcription and hsp70 protein synthesis have been well characterized in human cells[19]. The PGJ metabolite 15-deoxy-$\Delta^{12,14}PGJ_2$, which was shown to be the natural ligand for the adipocyte determination factor PPARγ inducing adipocyte differentiation[20], is also a potent inducer of HSF activation[21]. We have also shown that PGA_1 and PGJ_2 induce the synthesis of the oxidative stress protein haeme oxygenase in murine myoblasts[22] and in human monocytes from healthy donors (Elia et al., in preparation). Structure–activity relationship studies have recently shown that HSP induction requires the presence of a reactive α,β-unsaturated carbonyl group in the cyclopentane ring (cyclopentenone), which renders this portion of the molecule able to form Michael's adducts with cellular nucleophilics, and to covalently bind to cysteine residues of proteins[23].

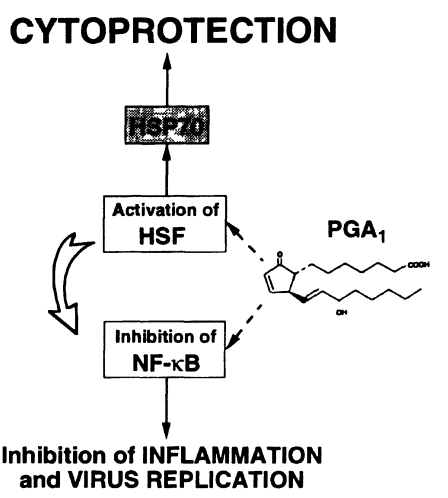
Induction of hsp70 by cyclopentenone prostaglandins is associated with a cytoprotective effect during hyperthermia and virus infection: cyclopentenone prostaglandins induce a thermotolerant state in human cells and protect cells from subsequent lethal injury[24]. Cyclopentenone prostaglandins are also characterized by a potent antiviral activity against a wide variety of DNA and RNA viruses, including the human immunodeficiency virus (HIV-1), in several experimental models in vitro and in vivo (reviewed in 25). A long-acting synthetic analogue of PGA_2 (di-M-PGA_2) has antiviral activity in a mouse model infected with influenza A virus[26]. In negative strand RNA virus models prostaglandins of the A and J type provoke a selective and dramatic block of viral protein synthesis[8,27]. This block is exerted at the translational level and is dependent on hsp70 expression in infected cells. A possible model has been hypothesized in which HSP and virus messages, both of which can be translated in conditions where cellular protein synthesis is impaired, could possess similar mechanisms for preferential translation, and could then compete with each other[25]. In the case of HIV-1 infection, a single treatment with PGA_1 or PGJ_2 is cytoprotective in lymphoblastoid CEM-SS cells, and causes a more than 1000-fold reduction in infectious virus yield, due to a selective block of HIV-1 mRNA transcription[28]. Inhibition of HIV-1 RNA transcription appears to be mediated by the recently discovered ability of cyclopentenone PGs to inhibit the activation of nuclear factor-κB (NF-κB)[21].

NUCLEAR FACTOR κ-B

NF-κB is an inducible eukaryotic transcription factor of the *rel* family, which normally exists in an inactive cytoplasmic complex, whose predominant form is a heterodimer composed of p50 and p65 (Rel A) subunits, bound to inhibitory proteins of the IκB family, usually IκBα, and is activated in response to primary (viruses, bacteria, UV) or secondary (inflammatory cytokines) pathogenic stimuli[29,30]. Stimulation triggers rapid phosphorylation and degradation of IκBα, resulting in NF-κB translocation to the nucleus, where it binds to DNA at specific κB-sites, rapidly inducing a variety of genes encoding signalling proteins. Target genes include cyclooxygenase-2, nitric-oxide synthase (iNOS), several inflammatory and chemotactic cytokines, cytokine receptors, cell adhesion molecules as well as viral genes[29]. NF-κB is a critical regulator of the immediate early pathogen response and activation of the immune system, and is involved in many pathological events, including progression of AIDS by enhancing HIV-1 transcription[31]. A possible role for NF-κB in atherosclerosis and reoxygenation damage has also been suggested[32,33]. Consequently NF-κB is an attractive therapeutic target for novel anti-inflammatory, antiviral and cytoprotective drugs, and the need for the development of effective NF-κB inhibitors with therapeutic efficacy is widely recognized.

Several arachidonic acid metabolites appear to interfere with NF-κB activation. 12(R)-hydroxyeicosatrienoic acid [12(R)-HETrE], but not the enantiomer 12(S)-HETrE, was found to markedly activate NF-κB DNA-binding activity in coronary microvessel endothelial cells[34]. 5-Lipoxygenase inhibitors (nordihydroguaiaretic acid and AA861), but not cyclooxygenase inhibitors, were reported to block IL-1β-induced expression of cell surface adhesion molecules (VCAM-1), via inhibition of NF-κB in human cells[35]. Leukotriene B_4 was also reported to activate NF-κB and NF-κB-controlled interleukin-6 expression in human blood monocytes[36]. However, we were unable to detect any significant increase in the constitutive level of DNA-binding activity of NF-κB in human peripheral blood monocytes in the presence of concentrations of LTB_4 ranging from 10^{-11} to 10^{-6} M (Elia G, Santoro MG, unpublished data). On the other hand, we have recently reported that cyclopentenone prostaglandins are potent inhibitors of NF-κB activation in human cells, and of NF-κB-dependent HIV-1 transcription in long terminal repeat-chloramphenicol acetyl transferase transient transfection experiments[21]. These eicosanoids act by inhibiting phosphorylation and preventing degradation of the NF-κB inhibitor IκBα[21]. Inhibition does not require protein synthesis and is dependent on the presence of a reactive cyclopentenonic moiety. Interestingly, the NF-κB inhibitory effect is tightly associated with HSF activation in human cells[21]. In fact activation of HSF1 by different cyclopentenone prostaglandins, by other chemical inducers, such as sodium arsenite, or by hyperthermia itself was found to prevent or suppress NF-κB activation, indicating a link between the regulatory pathways of these factors and suggesting the possibility that triggering of HSF1 could render cells unresponsive to stimulation of NF-κB. This hypothesis is very attractive considering the opposite roles of HSF and NF-κB in cytoprotection and cell injury respectively, as in the case of inflammation[14] and viral infection[8, 25, 31] (Figure 2).

Figure 2 Multiple targets for PGA_1 cytoprotective activity



PERSPECTIVES AND CONCLUSIONS

These observations encourage the search for novel prostanoids with the ability to induce HSP and inhibit NF-κB simultaneously, which could be utilized as cytoprotective and antiviral drugs. Prostaglandins are used clinically in the treatment of several diseases, including gastric ulcers and congenital heart disease, and to facilitate labour, and are generally effective and well-tolerated[37]. Administration of PGE_1 has also been shown to be beneficial in patients with fulminant viral hepatitis[38]. In studies on volunteers with hypertension, infusion with PGA_1 had beneficial effects on blood pressure, while no deleterious effects on kidney function or other significant side-effects were found[39]. On the other hand, the fact that different types of PGs are synthesized in different tissues throughout the body and have multiple effects on blood pressure, inflammatory and immune responses, poses several questions on the possible use of natural prostaglandins as cytoprotective compounds.

We have recently reported that one component of the PGA molecule, 2-cyclopenten-1-one, is able to activate HSF and to trigger rapid and selective transcription and translation of the *hsp70* gene, leading to the accumulation of high levels of hsp70 protein in human cells[23]. As previously shown for other HSP inducers, including antiviral prostaglandins, sodium arsenite, cadmium and hyperthermia itself, 2-cyclopenten-1-one-induced hsp70 synthesis is associated with a selective inhibition of virus protein synthesis, and viral replication[23]. The α,β-unsaturated carbonyl group present in 2-cyclopenten-1-one is the molecular structure responsible for HSF activation, since even high concentrations of cyclopentanone (a similar molecule with a saturated carbonyl), or cyclopentene, which contains a double bond, but not a carbonyl

group were unable to induce HSF DNA-binding activity. These results indicate that a class of novel molecules, characterized by the ability to activate HSF while inhibiting NF-κB, and devoid of the pleiotropic effects of natural PGs could be designed, opening new perspectives for therapeutic intervention in inflammatory and infectious diseases.

Acknowledgements

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