The Role of Nuclear Factor Kappa B Activation in Atherosclerosis and Ischemic Cardiac Injury

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ABSTRACT

The NF-κB family of transcription factors plays a critical role in many tissues by modulating both inflammation and cell survival, and this primarily comes about through transcriptional regulation of the downstream effector genes. This central role of coordinating complex programs of gene induction suggests that the NF-κB transcription factors and/or the signaling pathways leading to their activation may present a prime opportunity for performing therapeutic intervention. However, the dual role of this pathway in inflammation and survival dictates rigorous and empiric validation of such interventions in realistic models of disease before we can translate research findings to the clinical arena. Interestingly, the precise approach chosen to modulate NF-κB activation appears to dramatically alter the balance of the downstream effects on apoptosis and inflammation. Here we provide a brief overview of NF-κB signaling and its role in atherogenesis as well as in acute coronary syndromes, while considering the clinical implications for therapeutic strategies.

KEY WORDS: NF-κB; Atherosclerosis.

Introduction

Despite the impressive advances in diagnosis and patient management, acute coronary syndromes continue to be a major public health problem in the industrialized world and they are becoming an increasingly important problem in the developing countries. Our understanding of the mechanisms underlying atherogenesis and its sequelae such as unstable angina and acute myocardial infarction has been continuously evolving. Many lines of evidence suggest that inflammation plays a role in both the initial formation of atherosclerotic plaque and the rupture of these plaques, which are processes that underlie acute clinical presentations. Both processes are closely associated with inflammatory activation of the cells in the vessel wall and the cells circulating in the blood stream. In addition, although reperfusion of ischemic myocardium is undoubtedly the most important intervention to treat acute coronary syndromes, considerable evidence suggests that this may actually exacerbate ischemic injury through a process termed reperfusion injury; this appears to be mediated, at least in part, by the infiltration of inflammatory cells. The nuclear factor kappa B (NF-κB) family of transcription factors plays a central role in coordinating and regulating the expression of a wide variety of inflammatory genes that have been linked to all three of these cardiovascular pathologies. However, NF-κB controls not only inflammation, but also apoptosis in many cell types and; thus, therapeutic interventions aimed at inhibiting the function of NF-κB signaling may adversely affect cell survival. In this study, we here review the recent advances in our understanding of the NF-κB signaling, as well as the evidence linking NF-κB to atherogenesis, acute coronary syndromes, and reperfusion injury. Understanding the signaling mechanisms that control inflammation and the survival of cells in the cardiovascular system may lead to novel therapeutic strategies for treating intervention in these conditions.

NF-κB Signaling

NF-κB factors (p65 or RelA, p50, p52, c-Rel, and RelB) generally exist as homo- or heterodimers in the cytosol, and they are bound to one of three inhibitory, IκB subunits. Upon stimulation by a pro-inflammatory cytokine such as tumor necrosis factor (TNF)-α, β is activated and it phosphorylates IκB on two N-terminal serine residues. Phosphorylated IκB is degraded by the proteasome via polyubiquitination. The free form of NF-κB is translocated to the nucleus where it activa-
A viable alternative strategy is to utilize the expression of a kinase-inactive IKK-β mutant that functions as a dominant negative. Moreover, the latter approach may more closely model the effects one might expect with pharmacological inhibition of IKK-β. Dominant negative IKK-β (dnIKK-β) effectively abrogated NF-κB activation and inflammatory signaling in human endothelial cells in vitro. It is interesting that cell survival was not compromised under normal culture conditions.

It’s noteworthy that other mechanisms of NF-κB regulation also exist. Some members of the NF-κB family such as p65 can be regulated through direct phosphorylation of the transactivation domain (TAD), which appears necessary for full activation of gene transcription. In addition, NF-κB activation can also be regulated by tyrosine phosphorylation of IκB, and this leads to dissociation from p65 without IκB degradation. The kinase responsible for this event has not been identified, but it appears to be particularly important for NF-κB activation after hypoxia-reoxygenation. Finally, recent data suggests that reversible acetylation of p65 also modulates its association with IκB and transcriptional activity. Thus, multiple mechanisms of NF-κB regulation do exist and their relative contribution to cardiovascular disease is currently unknown.

A20 is an NF-κB-dependent zinc finger protein that appears to play a dominant role as an endogenous feedback inhibitor of NF-κB. Mice that lack A20 develop unchecked inflammation through sustained NF-κB activation and paradoxically, they also manifest increased apoptosis in response to specific stimuli. This suggests that A20 acts as an NF-κB inhibitor that actually suppresses apoptosis in contrast to many of the NF-κB inhibitors which potentiate cell death through the loss of NF-κB-dependent anti-apoptotic mechanisms. Indeed, expression studies suggest that this is the case and that A20 may work through inhibiting the interaction of TRADD and RIP with the TNF receptor complex. Whatever the mechanism, it is interesting and potentially of great practical utility to note that not all approaches to NF-κB inhibition result in increased cell death.

**Inflammation and NF-κB Activation in Atherosclerosis**

In a quiescent vessel, there is little inflammation and the normal vascular endothelium will allow little or no adhesion of leukocytes or platelets. However, a key step that occurs early in the pathogenesis of atherosclerosis appears to be activation of the endothelium in response to a variety of relevant stimuli, including oxidized lipids, cytokines or turbulent flow. Activated endothelium then expresses specific adhesion receptors and cytokines that enhance leukocyte recruitment into the vessel wall,
as well as factors that accelerate clot formation. Interestingly, recent evidence suggests that this inflammatory process is not confined to just one lesion in the coronary arteries, but that the process may be more generalized. Indeed, there is evidence that leukocyte inflammation can be found in multiple vascular beds, including the peripheral circulation.

Monocytes are the predominant leukocyte in atherosclerotic plaques and they may contribute to the development, progression and instability of atherosclerotic lesions. Recruitment of monocytes by the activated endothelium marks an early event in the formation of atherosclerotic lesions. Monocytes also appear to be important participants in the progression of atherosclerosis via their effects on inflammation, extracellular matrix remodeling and coagulation. Of note, genetically engineered mice that lack specific signals for the monocyte’s recruitment and function are resistant to atherosclerosis. Evidence from small clinical studies has also suggested that monocytes may be important in the plaque instability that is thought to underlie acute coronary syndromes.

Many of the molecules that are expressed by the activated vascular lesions are generally capable of enhancing leukocyte adhesion and particularly monocyte adhesion. These include both secreted molecules such as the chemokine family of chemoattractant cytokines and the surface-expressed cell adhesion molecules (CAMs) of the selectin (E-selectin and P-selectin) and immunoglobulin (ICAM-1 and VCAM-1) families.

The NF-κB family of transcription factors is involved in the regulation of many of these pro-inflammatory EC signals and they are activated both during the early stages of atheroma formation, as well as in advanced lesions. In addition, NF-κB also activates pro-coagulant (e.g. Tissue Factor) and proliferative targets (Cyclin D1) that could participate in atherogenesis. A striking correlation exists between the modulation of local NF-κB-activation and atherosclerotic plaque formation. Moreover, some studies suggest that NF-κB is activated in the peripheral monocytes of the patients suffering with unstable angina; this is consistent with prior reports showing the enhanced monocyte expression of the NF-κB-dependent genes and also the evidence of generalized systemic leukocyte activation in this setting.

However, the NF-κB-independent signaling pathways such as p38 and JNK/SAPK may also be important in vascular activation and, as noted above, NF-κB promotes a variety of potentially beneficial effects including endothelial survival and the expression of specific anti-oxidants. Whether NF-κB activation in any of the above mentioned cell types is an essential contributor to the pathogenesis of atherosclerosis or if it is simply a marker of inflammation has not been conclusively established.

**Inflammation and NF-κB Activation in Ischemic Injury**

One of the major sequelae of atherosclerosis is ischemic injury to the myocardium: this remains a major cause of morbidity and mortality in Western countries. The mainstay of current therapy for acute ischemia is reperfusion to the affected area via thrombolytic therapy or angioplasty. However, reperfusion may itself be associated with cardiac injury, and this is termed ischemia-reperfusion injury (IRI). While the net result of early reperfusion is beneficial, understanding the basis of reperfusion and learning to minimize the injury due to ischemia-reperfusion could maximize the benefits of reperfusion therapy for acute infarction. In animal models, cardiac IRI is associated with early endothelial dysfunction, an increased expression of adhesion molecules and cytokines, activation of the alternate complement pathway, infiltration of circulating neutrophils, release of oxygen free radicals as well as other potentially toxic products and programmed cell death.

However, the pathogenetic contribution of inflammation to ischemic injury is controversial. Several lines of investigation have suggested that inflammation may be an important functional contributor to the pathogenesis of myocardial injury after ischemia and reperfusion. For example, in animal models, neutrophil depletion with using antibodies, anti-metabolites or physical filtering, and inhibition of neutrophil adhesion with anti-CD18 mAb all of these substantially reduce injury after reperfusion. In addition, interventions that are targeted at a variety of specific inflammatory mediators have demonstrated benefits for IRI; these interventions include complement depletion or administering lipoxygenase inhibitors or antibodies to the pro-inflammatory cytokine, IL-1. Genetically engineered mice that lack TNF-α also have reduced injury after IRI. However, the benefits obtained in other studies by inhibiting inflammation-particularly for the “straight” infarction (without reperfusion)—have been less clear. Indeed, some investigators have suggested that inflammation may actually play a beneficial role in the healing process after infarction. In fact, some anti-inflammatory interventions such as administering corticosteroids have actually increased the size of infarcts and its complications.

However, given the pleiotropic effects of corticosteroids, it is difficult to ascribe these adverse effects to a specific cause. It is noteworthy that this disappointing clinical result with administering corticosteroids occurred in the pre-thrombolytic era and it presumably reflects...
the primarily outcomes in occluded vessels.

As part of this inflammatory process, multiple studies have demonstrated that NF-κB is activated in IRI. While cardiomyocytes appear to require NF-κB signaling for survival, at least under some circumstances, many studies have suggested that inhibition of NF-κB can have beneficial effects during the acute phase of IRI. Such interventions have involved the expression or delivery of a peptide that inhibits proteosomal degradation of IκB or the acute delivery of "decoy oligonucleotides" that competitively inhibit NF-κB DNA binding. In many of these studies, it is not clear to what extent NF-κB is being inhibited in cardiomyocytes as opposed to NF-κB inhibition in endothelial cells or other cells that might play a role in initial leukocyte recruitment. Moreover, the majority of studies on both sides of this issue have examined only the relatively acute endpoints. Validation of the targets for potential future clinical intervention will obviously require that any of the observed benefits be sustained over time and that they are not outweighed by the possible adverse side effects.

It seems likely that rather than being entirely beneficial or deleterious, inflammation probably plays multiple roles in the injury and recovery after IRI or infarction. Thus, the timing and precise nature of any anti-inflammatory interventions may be critical determinants of the outcome. For example, it’s possible that the roles of the early, predominantly neutrophil infiltration may be quite different from the later, predominantly mononuclear infiltrate in IRI. Moreover, as noted above, not all approaches to NF-κB inhibition result in an identical phenotype, particularly with respect to such important effects as cell survival. Finally, the balance of the NF-κB-dependent effects may differ for specific cell types and so play different roles in endothelial cells and cardiomyocytes. These issues were elegantly illustrated in a recent study of IKK-β signaling and NF-κB activation in a murine model of intestinal ischemia-reperfusion injury. Enterocyte-specific deletion of IKK-β prevented the systemic inflammatory response to bowel ischemia-reperfusion; however it potentiated apoptotic injury in the intestinal mucosa. It is currently unclear what the long-term, net effect of ablating IKK-β signaling in cardiomyocytes, endothelial cells or other cell types would be for cardiac ischemic syndromes or indeed the atherosclerosis that leads to them. Conditional or tissue-specific deletion of IKK-β provides a powerful tool to specifically and effectively abrogate IKK-β signaling, and this will enable such questions to be examined more rigorously.

**Pharmacological Inhibitors of NF-κB**

Multiple pharmacological approaches have been developed for inhibiting NF-κB. For example, some of these approaches that block the proteosomal degradation of IκB, are likely to affect many other signaling pathways that rely on this common regulatory mechanism. Since IKK-β appears to be the critical kinase required for NF-κB activation and since no other targets of IKK-β have been identified to date, it is a logical candidate for performing pharmacological manipulation of this pathway. Interestingly, aspirin and salicylate have been reported to bind to and inhibit IKK-β. While it may be tempting to speculate that some of the benefits of administering aspirin after reperfusion or in other settings could be derived from the inhibition of IKK-β, both the pleiotropic effects of aspirin and the relatively low doses that are administered clinically in these settings suggest that other mechanisms may be the

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**Table 1. Various strategies of NF-κB inhibition for intestinal ischemia-reperfusion injury**

<table>
<thead>
<tr>
<th>Models &amp; NF-κB modulators</th>
<th>Outcomes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary artery occlusion, transfection of NF-κB decay oligodeoxynucleotides</td>
<td>Infarct size ↓</td>
<td>Morishita et al. (1997)</td>
</tr>
<tr>
<td>Pig, LAD-occlusion and reperfusion, retroinfection of liposomal NF-κB decay oligonucleotides</td>
<td>Infarct size ↓, Recovery of myocardial function ↑</td>
<td>Kupatt et al. (2002)</td>
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<tr>
<td>lnBz J/N mice (defect in activation of NF-κB), LAD ligation</td>
<td>Infarct size ↓</td>
<td>Misra et al. (2003)</td>
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<tr>
<td>Apoptotic nuclei ↑</td>
<td>Suardito et al. (2003)</td>
<td></td>
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<tr>
<td>Mouse, total occlusion of the coronary artery and reperfusion, recombinant adeno-associated virus encoding the gene for the lnBz</td>
<td>Infarct size ↓</td>
<td></td>
</tr>
<tr>
<td>Rat, ischemia of left cremaster muscle and reperfusion, NF-κB inhibitor PTC</td>
<td>InNOS expression ↑</td>
<td>Qi et al. (2004)</td>
</tr>
<tr>
<td>Rat, LAD ligation and reperfusion, NF-κB inhibitor IMD-0354</td>
<td>Blood flow ↑</td>
<td></td>
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<tr>
<td>Rat, LAD ligation and reperfusion, recombinant adenovirus mediated overexpression of lnBz</td>
<td>Infarct size ↓, Cardiac function ↑</td>
<td>Onai et al. (2003)</td>
</tr>
<tr>
<td>Rat ventricular myocytes, hypoxia for 24 hours</td>
<td>ICAM-1 and P-selectin expression ↓</td>
<td></td>
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<tr>
<td>Recombinant adenosine encoding IKKβ wt and IKKβ mt</td>
<td>Cardiac function (%FS, %EF) ↑</td>
<td>Tresch et al. (2004)</td>
</tr>
<tr>
<td>Rat, LAD ligation and reperfusion, NF-κB inhibition by Ad5-IκB-IκB</td>
<td>NF-κB activation with IKKβ wt expression → hypoxia-induced cell death ↓</td>
<td>Regula et al. (2004)</td>
</tr>
<tr>
<td>Rat, LAD ligation and reperfusion, NF-κB inhibition by Ad5-ΔnMyD88</td>
<td>Infarct size ↓</td>
<td>Hua et al. (2003)</td>
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<tr>
<td>Cardiac myocyte apoptosis ↓</td>
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NF-κB: nuclear factor kappa B, LAD: left anterior descending artery, PTC: pyrrolidine-1,4,5-triazole, IMD-0354: N-(4,5-Bis-trifluoromethyl-phenyl)-5-chloro-2-hydroxy-benzamid, IKKβ: IκB kinase, iNOS: inducible nitric oxide synthase, ICAM: intercellular adhesion molecule, %FS: fractional shortening, %EF: ejection fraction
primary contributors. Nevertheless, this finding provides a structural precedent for the development of other small-sized molecular inhibitors. An IKK-β inhibitor was recently described that had favorable pharmacokinetics and efficacy after intravenous or oral administration in an animal model. Such reagents should facilitate investigating the role of IKK-β and NF-κB signaling in disease models, as well as determining their true potential as therapeutic targets. Of note, since no tissue-specific isoforms of IKK-β have been identified, systemic delivery of such inhibitors may also have other diverse effects on multiple organs that will need to be carefully examined. Transcriptional decoys or double-stranded DNA oligonucleotides encoding an NF-κB-binding site have been effectively used to inhibit NF-κB in animal models. Since the NF-κB binding sequence is somewhat variable, this approach offers the possibility of specifically inhibiting only a subset of the downstream NF-κB targets, although this has not yet been experimentally demonstrated.

**Conclusions and Clinical Implications**

Many studies have suggested that NF-κB is activated either locally or systemically in atherosclerosis, acute coronary syndromes and cardiac ischemia-reperfusion injury (Table 1). Given the plethora of NF-κB-dependent transcripts that have been previously implicated in these syndromes, it seems likely that NF-κB activation contributes to the pathophysiology of these syndromes, although this has not been conclusively demonstrated. The central role of NF-κB makes it an appealing focus for intervention, but this also carries a significant risk for such adverse consequences as potentiation of apoptosis or unintentional disruption of signaling in the non-target tissues. It seems likely that the timing, duration and mechanism of NF-κB inhibition will be the critical determinants of success of these intervention strategies. The complexity of the NF-κB interactions suggests that these issues will need to be addressed empirically in realistic models of disease before we can consider translating research findings into clinical applications. Nevertheless, as our understanding of NF-κB signaling increases, there is reason for cautious optimism that specific and well-tolerated strategies will be devised to exploit this pathway for achieving a significant therapeutic advantage.

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