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Review Article

The role of NF-κB in the pathogenesis of heart failure and endotoxemia

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CONTENTS

Abstract

Nuclear factor-κB (NF-κB), a transcription factor mainly involved in stress-induced, immune and inflammatory responses, plays a critical role in the pathogenesis of several human diseases. It is activated in heart failure and endotoxemia, and its inhibition is associated with therapeutic effects in these conditions. NF-κB is also activated by angiotensin II, which can be inhibited using an angiotensin II receptor antagonist. Pimobendan, a phosphodiesterase type 3 (PDE3) inhibitor, suppresses the activation of NF-κB and improves the long-term prognosis in heart failure.

Introduction

There is increasing evidence that the inflammatory response is associated with an increase in the transcription of inflammatory genes. Gene transcription is regulated by transcription factors that are sequence-specific DNA-binding proteins capable of modulating the rate of transcription. One such transcription factor, nuclear factor-κB (NF-κB), regulates the expression of a wide range of genes involved in immune and inflammatory responses. NF-κB belongs to a family of proteins, including p50, p52, RelA, c-Rel and RelB, which can form transcriptionally active or repressive homo- or heterodimers. In its inactive state, an NF-κB dimer is present in the cytosol, where it is bound to an inhibitory protein such as IκB (1).

The NF-κB family of transcription factors is mainly involved in stress-induced, immune and inflammatory responses. These molecules also play important roles during the development of certain hematopoietic cells, keratinocytes and lymphoid organ structures. More recently, members of the NF-κB family have been implicated in neoplastic progression and in the formation of neuronal synapses. These important and diverse functions make NF-κB one of the most studied transcriptional factors in biology. Members of this family are formed by dimeric combinations of subunits and are activated by several receptor-mediated signaling pathways. Once activated, they stimulate transcription-specific sets of target genes mediating a multitude of diverse functions (2).

NF-κB plays a critical role in the pathogenesis of several human disorders, particularly those with an inflammatory component. Examples of genes dependent on the activation of NF-κB include: 1) the cytokines tumor necrosis factor (TNF)-α and interleukins IL-6, IL-8 and IL-1β; 2) the adhesion molecules E-selectin, intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1); and 3) the enzymes nitric oxide synthase (NOS) and cyclooxygenase type 2 (COX-2) (3, 4). The expression of IL-1β, TNF-α and inducible NOS (iNOS) is regulated at the transcriptional level and the activation of NF-κB is one of the key factors (5).

Therefore, the inhibition of NF-κB activation may have broad therapeutic applications. Several inhibitors have recently been described, most of which are effective in cell-based experiments *in vitro*, although they are unsuitable for therapeutic use since they cause unacceptable adverse effects (6). This review describes the effects of drugs which inhibit the activation of NF-κB in congestive heart failure (CHF) and endotoxemia.

Role of NF-κB in inflammatory disorders

The importance of NF-κB in regulating the expression of proinflammatory cytokines, cell adhesion molecules and enzymes strongly suggests that NF-κB inhibitors would exert potent and broad-based antiinflammatory activity in several human diseases. Consistent with this

expectation, glucocorticoids are highly effective antiinflammatory agents and the activity is primarily attributable to the inhibition of NF-κB-mediated transcription (7). While the use of glucocorticoids is limited by adverse effects unrelated to their anti-NF-κB properties, drugs that inhibit NF-κB specifically may be expected to have glucocorticoid-like effects without the undesirable toxicity of glucocorticoids.

That NF-κB inhibitors would be effective antiviral agents may seem paradoxical, since the NF-κB pathway is important in both innate and adaptive immunity (8, 9). However, many viruses utilize NF-κB to ensure their own proliferation. For example, the HIV, herpes, hepatitis C and encephalomyocarditis (EMCV) viruses exploit the regulation of antiapoptotic factors by NF-κB, since this prolongs the survival of the host cells, giving the virus more time for replication (10). In addition, viruses such as HIV-1 and herpes simplex virus type 1 (HSV-1) require host NF-κB for their transcription (11, 12). Indeed, RNA interference directed against p65 markedly reduced the replication of HIV-1 in MAGI cells (13). These observations suggest that NF-κB may be an appropriate target in the treatment of some viral diseases.

Inflammation and cytokines are increasingly recognized as important factors in the pathogenesis and pathophysiology of CHF and other cardiac disorders. High levels of circulating cytokines, which depress myocardial contractility *in vitro* and *in vivo*, have been reported in patients with CHF (14-16). There is increasing evidence that the inflammatory response and the production of cytokines are associated with the activation of NF-κB. The latter is activated by several factors which increase the inflammatory response, including viral infections, oxidants and antigens. This activation, in turn, coordinates the expression of several protein-encoding genes, such as cytokines, chemokines, adhesion molecules and enzymes involved in mediator synthesis, and further amplifies and perpetuates the inflammatory response. NF-κB is therefore clearly a target for new antiinflammatory interventions.

Role of NF-κB in heart failure

Chronic CHF, where the heart is unable to pump enough blood to support other organs, may develop as a result of chronic hypertension, ischemia and infection. A dominant mechanism behind the development of CHF is long-standing cardiac hypertrophy. NF-κB is essential for the induction of cardiomyocyte hypertrophy by G-protein-coupled receptors, (17, 18) and sustained activation of NF-κB has been observed in a rat model of CHF (19).

We have reported that circulating TNF-α levels are increased in mice with CHF infected with EMCV, and that pretreatment with an anti-TNF-α antibody attenuated myocardial injury and decreased mortality in the acute stage (20). In the same model, we also observed that the intracardiac expression of cytokine genes was increased to various degrees proportional to the severity of the disease (21). A recent study of EMCV myocarditis has reported a higher survival of TNF-α knockout than in control mice (22). Since knockout mice lack TNF-α during their development, their response to viral infection may be different than that associated with the specific neutralization of TNF-α immediately after viral infection.

Phosphodiesterase (PDE) inhibitors, originally developed to treat CHF, can inhibit the production of cytokines, and different inhibitors produce different effects. In our murine model of CHF caused by viral myocarditis, pimobendan prolonged survival, attenuated inflammatory lesions and decreased the production of intracardiac IL-1β, IL-6, TNF-α and nitric oxide (NO) (23). More recently, pimobendan was shown to improve quality of life and decrease adverse cardiac events in patients with CHF (24). In a study from our laboratory, pimobendan, but not other PDE3 inhibitors, blocked the activation of NF-κB (Fig. 1) (25). Thus, inhibition of the production of proinflammatory cytokines and NO by pimobendan can be explained by its inhibitory effect on the activation of NF-κB, which also partially explains the effects of pimobendan in CHF. Amrinone, another PDE3 inhibitor, also attenuates the activation of NF-κB in cardiomyocytes (26).

Most viruses encode proteins capable of activating NF-κB. Mice lacking the p50 subunit of NF-κB are resistant to EMCV infection, and fibroblasts from mice lacking p50 have an enhanced induction of interferon beta transcription upon infection with EMCV. Therefore, the p50 subunit of NF-κB may downregulate transcriptional responses that have important consequences on the *in vivo* response to pathogens (27). In our experiments, EMCV activated NF-κB in cultured noncardiac but not cardiac myocytes. For reasons which remain unclear, the activation of NF-κB does not seem to be a major pathway in myocytic EMCV infection. In a murine model of EMCV-induced myocarditis, SUN-C8079, a new NF-κB inhibitor, reduced mortality, attenuated myocardial necrosis and cellular infiltration, and decreased the intracardiac production of IL-1β and TNF-α, without significantly changing viral replication (28). Although glucocorticoids and ciclosporin inhibit the activation of NF-κB, both enhance viral replication and exacerbate myocardial injury in acute EMCV myocarditis (29, 30). Inducible NOS also has an NF-κB response site in its promoter region (31). In an earlier study, we found that EMCV infection increases the production of NO in macrophages, and that its inhibition attenuates the pathological manifestations of EMCV myocarditis (32).

Angiotensin II activates NF-κB

Angiotensin II plays an important role in the pathogenesis of CHF, and angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers have clear therapeutic effects in patients suffering from CHF (33). Angiotensin-converting enzyme inhibitors also attenuate virus-induced myocardial injury, one of the causes of CHF (34). In a classic murine model of acute

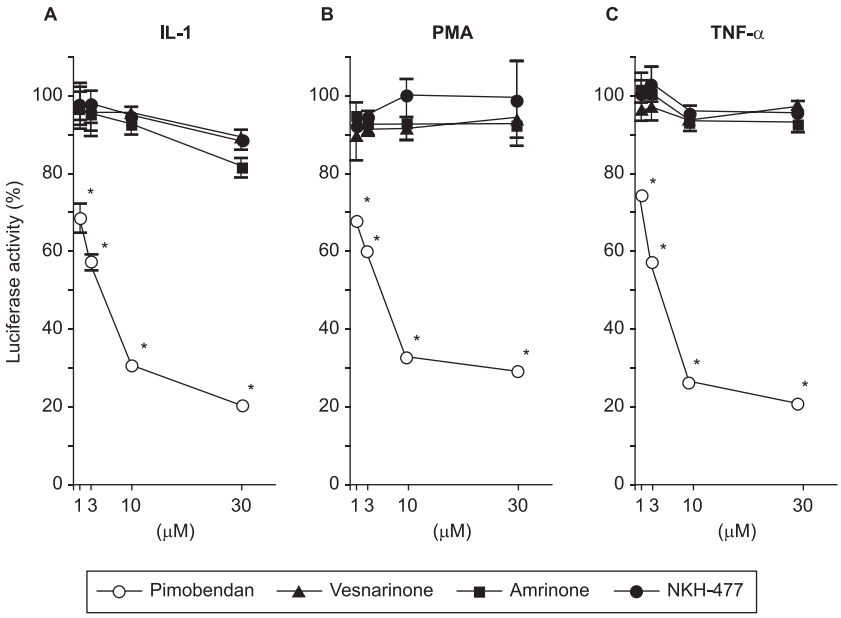

Fig. 1. Effects of pimobendan, vesnarinone, amrinone and NKH-477 on transcriptional responses of NF-κB stimulated with IL-1 (A), PMA (B) and TNF-α (C). *$p < 0.0001$. Reproduced with permission from Ref. 25.

viral myocarditis, we found that inoculation with EMCV increased the plasma concentrations of angiotensin II, and that myocardial lesions were attenuated by the administration of an angiotensin II receptor blocker (35). However, the downstream events responsible for the angiotensin II-induced myocardial injury remain to be clarified. Growing attention is being paid to the regulation and function of transcription factors such as NF-κB and activator protein-1 (AP-1) during tissue injury. Angiotensin II activates various nuclear transcription factors, including the signal transducer and activator of transcription (STAT) factors, AP-1, NF-κB and the cAMP response element-binding protein (CREB). Among these, NF-κB is of special interest, as it plays a pivotal role in the control of several genes, including cytokines, chemokines, adhesion molecules, COX-2 and iNOS, as described earlier. NF-κB is activated by various stimuli, including transcription factors, cytokines, hypoxia, free radicals and oxidants, LPS, activators of protein kinase C (PKC), ultraviolet radiation and viruses, while its inhibition prevents inflammatory responses and attenuates the expression of proinflammatory cytokines.

The important role played by cytokines in the pathophysiology of viral myocarditis was recently emphasized. Angiotensin II is capable of producing inflammatory changes such as vascular inflammation and chemotaxis of immune cells by signals through the AT_1 receptor (36, 37). *In vitro*, angiotensin II induces the expression of TNF-α and IL-6 in cardiac fibroblasts, and transforming growth factor-β (TGF-β) in cardiomyocytes (38). Furthermore, ACE inhibition decreases the expression of serum TNF-α induced by LPS administration in mice.

In a recent study from our laboratory, angiotensin II and viral infection activated NF-κB and increased the expression of TNF-α and IL-1β in the heart of normal mice, but not in AT_1 receptor knockout mice (Fig. 2) (39). These observations suggest that AT_1 receptor signaling is important in the development of virus-induced myocardial injury via the proinflammatory angiotensin II and NF-κB/cytokine pathway. Furthermore, aldosterone also activates NF-κB and increases the expression of cytokines and adhesion molecules (40). Thus, the renin-angiotensin-aldosterone system may play an important role in inflammation (41).

Role of NF-κB in endotoxemia

Exposure to a sublethal dose of LPS protects mice from subsequent challenges with doses otherwise lethal to naïve animals. Alternatively, spliced forms of MyD88, the proximal adaptor molecule involved in the IL-1 receptor (IL-1R)- and Toll-like receptor (TLR)-induced activation of NF-κB, were characterized. MyD88 contains a *C*-terminal TIR (Toll/IL-IR) homology domain and an *N*-terminal death domain (DD). The TIR domain mediates binding to the receptor, whereas the DD recruits IL-1R-associated kinase 1 (IRAK1). An intermediate domain of MyD88 has been found to mediate the recruitment of IRAK4. This positioning leads to the phosphorylation of IRAK1 by IRAK4, triggering the dissociation of IRAK1 from the receptor and subsequent activation of the IKK signalosome. A splice variant of MyD88, named MyD88S, is expressed in the spleen and is highly LPS-inducible

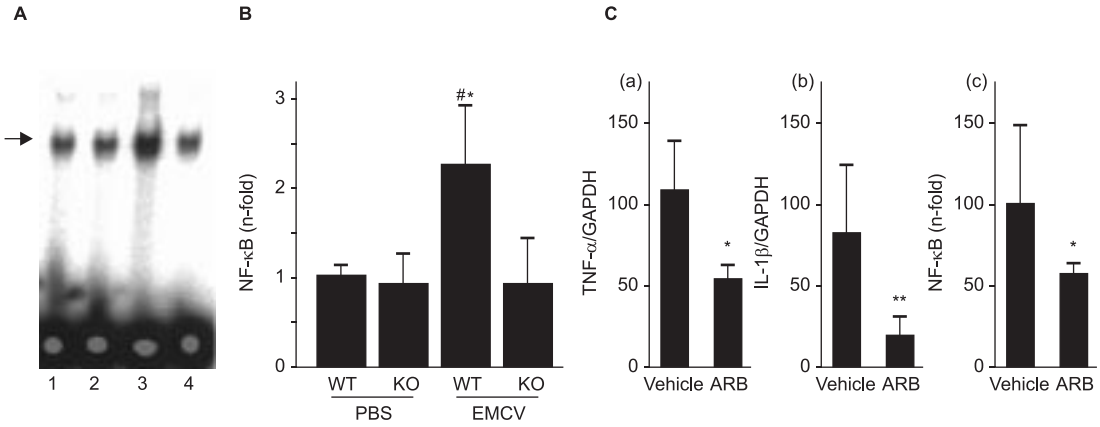

Fig. 2. Activity of NF-κB in heart tissue after EMCV-induced myocarditis. A) Representative electrophoretic mobility shift assay (EMSA) experiment. Lane 1 = wild-type (WT) mice with phosphate-buffered saline (PBS) inoculation; lane 2 = knockout (KO) mice with PBS inoculation; lane 3 = WT mice with EMCV inoculation; lane 4 = KO mice with EMCV inoculation. B) Densitometric analysis of the results expressed as arbitrary units of mean values ± SEM of 8 animals from each group. The mean value of NF-κB activities in the WT group with PBS inoculation is represented as 1. *$p < 0.01$ *vs.* WT with PBS inoculation. #$p < 0.01$ *vs.* KO with EMCV inoculation. C) Significant attenuation of proinflammatory cytokine expression and NF-κB activity in the heart of WT mice with viral myocarditis by angiotensin II type 1 receptor blockade (ARB) with candesartan (10 mg/kg/day). Myocardial cytokine mRNAs of TNF-α (a) and IL-1β (b). (c) Densitometric analysis of the results expressed as arbitrary units of mean values ± SEM. Mean NF-κB activity in the infected WT mice treated with the vehicle is represented as 1. *$p < 0.01$; **$p < 0.05$. Reproduced with permission from Ref. 39.

(42). Overexpression studies revealed that MyD88S can function as a dominant-negative inhibitor of IL-1- and LPS-induced NF-κB activation. MyD88S lacks the intermediate domain, such that the adaptor remains capable of binding IL-1R and IRAK1, but is unable to recruit IRAK4. IRAK phosphorylation does not occur and the activation of NF-κB is aborted. In the case of a mouse treated with a sublethal dose of LPS, dominant-negative MyD88S may be induced, which could render cells refractory to further challenge by LPS (1).

In endotoxemia, the acute and overwhelming activation of NF-κB can cause widespread endothelial cell death with disturbances in membrane permeability and disseminated coagulation (43). However, it has become apparent that NF-κB can also protect cells against death. TNF-α can cause apoptosis, which is often accompanied by an increased activation of NF-κB. However, in some experiments, the inhibition of NF-κB increased rather than decreased the rates of cell death (44). In addition, NF-κB is activated during ischemic preconditioning, and its pharmacological inhibition attenuated cardioprotection in animal models (45). It is apparent that NF-κB plays multiple roles in the regulation of cell viability and that much additional work is needed to fully clarify all of them (46).

Glucocorticoids are effective inhibitors of NF-κB, although they cause endocrine and metabolic adverse effects when prescribed systemically, which might not be the case with a more specific inhibitor. Aspirin and sodium salicylate also inhibit the activation of NF-κB, albeit only in relatively high concentrations (47), and gold salts inhibit the binding of NF-κB to DNA (48), suggesting that the antiinflammatory properties of these drugs may be mediated, at least in part, by inhibition of NF-κB. Pyrrolidine dithiocarbamate inhibits the activation of NF-κB and protects against LPS-induced shock (49, 50), but it was also found to be toxic (6).

We have reported that various PDE inhibitors differentially inhibit the production of cytokines by human peripheral blood mononuclear cells stimulated by LPS (51). It is noteworthy that most PDE inhibitors, except 8-Br-cAMP, inhibited the production of cytokines. We also studied the effects of various PDE3 inhibitors on the production of NO in LPS-activated macrophages (52). Whereas the production of NO was strongly inhibited by pimobendan, it was increased by 8-Br-cAMP. Thus, the inhibitory effects of pimobendan on the production of proinflammatory cytokines and expression of the iNOS gene can not be explained by an increase in cAMP. The effects of pimobendan were ultimately attributed to inhibition of NF-κB (25).

We have recently shown that SUN-C8079, a new NF-κB inhibitor, inhibits the activation of NF-κB by LPS *in vitro*, and that its administration to mice suppresses the production of LPS-induced TNF-α protein and prolongs survival in a dose-related manner (Fig. 3) (28). Therefore, the activation of NF-κB *in vivo* appears to be a key mediator of the injury induced by LPS in multiple organs, and inhibiting this activation may represent a novel strategy in the treatment of sepsis-induced multiple organ injury. The mechanism of NF-κB inhibition seems to occur downstream of signal transduction in the nucleus, since the effect of SUN-C8079 was observed as late as 3 h after stimulation with IL-1β or TNF-α. However, it did not block the direct binding of NF-κB to the NF-κB consensus oligonucleotide. Therefore, we hypothesize that a nuclear

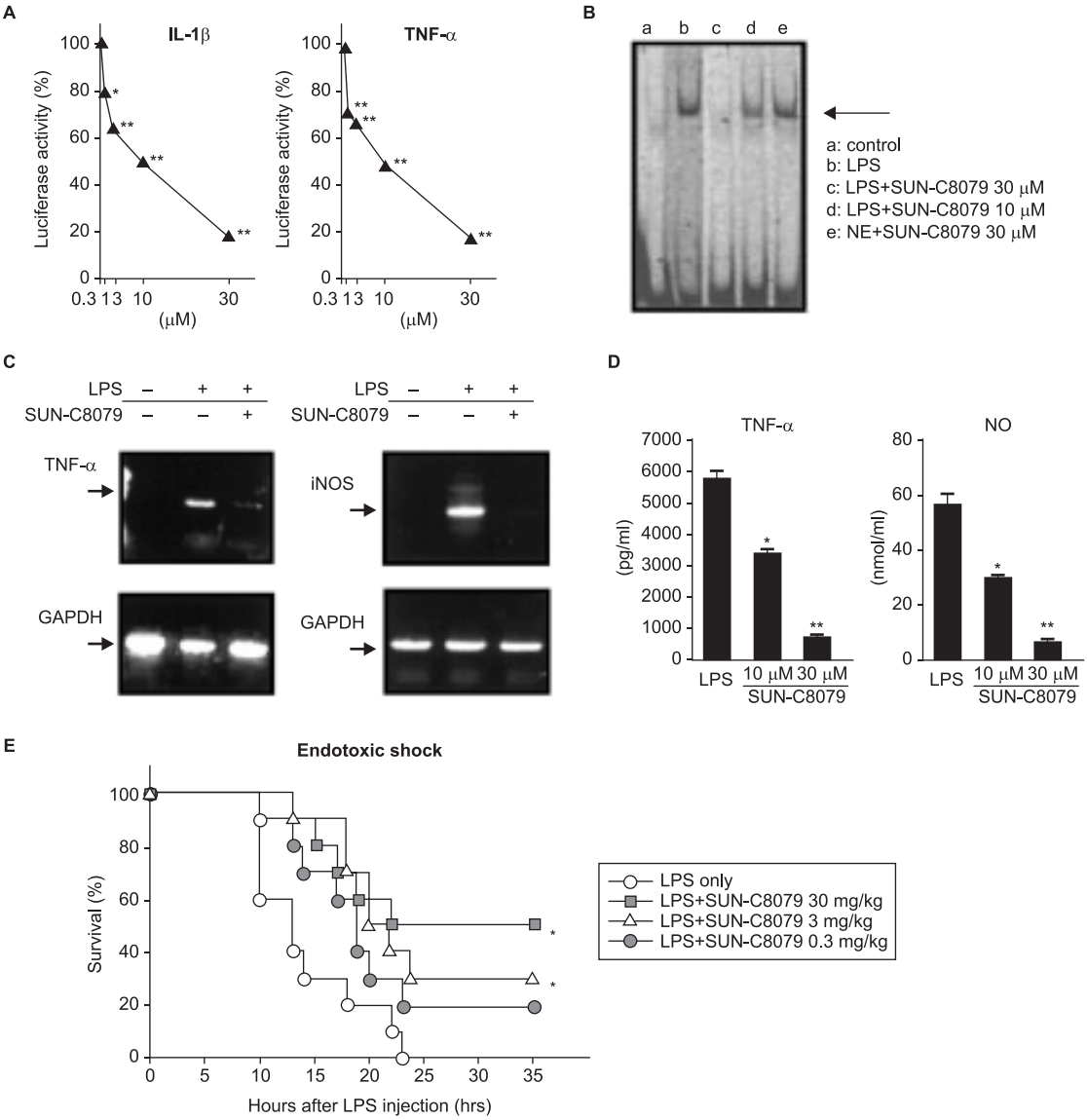

Fig. 3. A) Effects of the NF-κB inhibitor SUN-C8079 on the transcriptional responses of NF-κB stimulated with IL-1β (1 ng/ml) and TNF-α (50 ng/ml). *$p < 0.001$; **$p < 0.0001$. B) Effects of SUN-C8079 on LPS-stimulated NF-κB activation in the macrophage cell line RAW 264.7 in an electrophoretic mobility shift assay (EMSA). Lane a = vehicle without LPS; lane b = vehicle + LPS (10 μg/ml); lane c = LPS (10 μg/ml) + SUN-C8079 (30 μM); lane d = LPS (10 μg/ml) + SUN-C8079 (10 μM); lane e = nuclear extracts (NE) + SUN-C8079 (30 μM). No inhibitory effect of SUN-C8079 on the direct binding of NF-κB to NF-κB consensus oligonucleotide. Lanes a-e contain 1% DMSO. Results are representative of 3 separate experiments. C) Effects of SUN-C8079 on the gene expression of TNF-α and iNOS stimulated with LPS. *$p < 0.001$; **$p < 0.0001$. D) Effects of SUN-C8079 (10 or 30 μM) on the production of TNF-α protein and nitric oxide (NO) stimulated with LPS. E) Effects of SUN-C8079 on LPS-induced lethal toxicity in mice. *$p < 0.01$ for LPS + SUN-C8079 (0.3, 3 or 30 mg/kg) *vs.* LPS alone. Reproduced with permission from Ref. 28.

factor necessary to activate NF-κB is functionally targeted by SUN-C8079. Although we did not observe an antioxidant effect for SUN-C8079 in an *in vitro* system generating superoxide anion, antioxidant effects on other free radicals can not be excluded. Studies are under way in our laboratory to identify the target(s) of SUN-C8079 inhibition.

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