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Review Article

Necroptosis – a novel cell death mechanism

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Abstract

A comprehensive mechanistic understanding of the apoptotic cell death pathway has led to intervention strategies (*e.g.*, caspase inhibitors) that showed promise in attenuating tissue injury in animal models of human disease. Very few attempts, however, have been made to develop therapeutics specifically targeting necrosis due to the conventional notion that necrotic cell death is a nonregulated response to overwhelming stress. This belief is directly challenged by recent studies demonstrating the existence of regulated caspase-independent cell death mechanisms with morphological features resembling necrosis. Regulated necrosis pathways, such as necroptosis, may offer unprecedented opportunities to selectively target pathological necrotic cell death in a variety of diseases. This review highlights the structure-activity relationship (SAR) studies of four distinct classes of necroptosis inhibitors. Optimization of other properties (*i.e.*, metabolic stability) necessary for providing useful pharmacological tools for *in vivo* evaluation is also illustrated for several of the inhibitor series, and potential clinical indications that might be amenable to disease-modifying therapy utilizing necroptosis inhibitors (*i.e.*, necrostatins) are also presented.

Introduction

Cell death is a fundamental biological process that occurs and in certain instances is necessary throughout an organism's life cycle. For example, during development some tissues may only serve a transient role. Once their function is complete, cell death ensues, allowing their efficient removal as part of normal maturation. Similarly, cell death serves to eliminate cells throughout adulthood. For example, following an immune response to foreign antigens, subclasses of lymphocytes are cleared from the circulation once their function has been completed. In addition, throughout an organism's life span, some tissues, such as epithelial cells lining the villus tips of the small intestine and the luminal surface of the large intestine, are continuously replenished by way of processes involving controlled and selective cell death. Along with the important physiological functions of cell death, its aberrant regulation can lead to pathological conditions that affect human health. Most of the resulting ailments currently receive only symptomatic treatment as opposed to therapies that would slow or halt the underlying pathological processes. Only with a more complete understanding of the complex mechanisms of cell death and the role that these processes play in organ dysfunction will disease-modifying therapies become more readily available.

Morphological and mechanistic characteristics of cell death

Historically, cell death has been classified according to morphological characteristics. Based on these criteria, cell death typically has been described as either apoptotic or necrotic (1). However, as accumulating evidence clearly suggests, such nomenclature does not adequately reflect a mechanistic understanding of cell death and provides limited guidance for designing therapeutic strategies aimed at the development of cell death inhibitors. Of these two morphological forms of cell death,

apoptosis has received considerable attention, which has resulted in a more complete mechanistic understanding of this process. In contrast, necrotic cell death has generated much less interest, due in part to the conventional notion that it is a passive and unregulated consequence of overwhelming stress. In certain circumstances this is indeed the case. However, as we will discuss below, this situation may not be universally true for all conditions that ultimately present morphologically as cellular necrosis.

Apoptosis

Apoptosis is a genetically encoded cell death mechanism that has been clearly established to be required for both development and tissue homeostasis (2, 3). Extensive studies on apoptosis during the past decade revealed that it is executed through a highly regulated sequence of steps, including cytochrome *c* release from mitochondria regulated by the Bcl-2 protein family, formation of a cytosolic apoptosome complex and caspase activation, which lead to downstream events, such as membrane blebbing, DNA fragmentation and expression of cell-surface signals, to facilitate removal of dead cells. Cells eventually fragment into apoptotic bodies that are efficiently removed through the process of phagocytosis, primarily by macrophages in the periphery and microglia in the central nervous system (CNS). This elegant and complex process eliminates dead cell debris and prevents a host inflammatory response. However, aberrant apoptosis regulation (including suppression or induction) may underlie the etiology of many human diseases. For example, in neoplasms a cell's ability to undergo apoptosis is suppressed, resulting in an inability to counteract abnormal cellular proliferation. In other diseases, erroneous activation of apoptosis may contribute to acute or chronic tissue injury.

Since caspases play a pivotal role in the execution of apoptosis, the development of caspase inhibitors has become a key strategy for the treatment of diseases where flawed apoptotic cell death activation is operative. Caspases belong to an enzyme family of cysteine proteases that present attractive molecular targets for inhibition by small molecules (4-6). However, the clinical development and regulatory approval of caspase inhibitors have faced several challenges, including difficulty in designing molecules that simultaneously possess potent caspase-inhibitory activity and have adequate pharmaceutical properties (*i.e.*, solubility and tissue distribution). In addition, since apoptosis is required for normal physiology, inhibitors could cause unwanted side effects by disrupting nonpathological processes. Despite these challenges, the development of caspase inhibitors continues to be an appealing strategy and an ongoing pursuit within many drug discovery organizations.

Necrosis

Necrosis represents a type of cell death morphologically and mechanistically distinct from apoptosis. Necrosis is characterized by rapid mitochondrial dysfunction, oxidative stress, disruption of calcium homeostasis and organelle swelling followed by cell lysis, which is accompanied by a host inflammatory response (7). Although in recent years multiple studies have demonstrated apoptosis activation in various diseases, necrosis remains the prevalent form of acute cell death in many pathologies, including cerebral ischemia (8), myocardial infarction (9), trauma and possibly some forms of neurodegeneration (10). Very few attempts, however, have been made to develop therapeutics specifically targeting necrosis because of the conventional notion that, unlike apoptosis, necrotic cell death is a nonregulated response to overwhelming stress.

Necroptosis: a form of regulated necrosis

The notion that all necrotic cell death is nonregulated has been directly challenged by a number of recent studies demonstrating the existence of regulated caspase-independent cell death mechanisms (11-18) with morphological features resembling necrosis. Previously, we have defined one type of regulated necrosis, termed necroptosis (19). This form of cell death can be initiated by various stimuli (*e.g.*, TNF-α and Fas ligand) in an array of cell types (*e.g.*, monocytes, fibroblasts, lymphocytes, macrophages, epithelial cells and neurons) and its activation is specifically mediated by the kinase activity of a death receptor-binding molecule, RIP1 (20). RIP1 triggers downstream execution events through still poorly understood mechanisms, including induction of mitochondrial dysfunction, oxidative stress and autophagy (21-23). Importantly, the resulting cell death is morphologically indistinguishable from nonregulated cellular necrosis (19, 20).

Accumulating evidence suggests that, while apoptosis may be best suited for normal physiological regulation, necrotic cell death becomes a predominant form of death under conditions of excessive stress and acute energy loss characteristic of many human diseases (24). The conventional view is that apoptosis is actively executed from within the cell and that necrosis is an uncontrollable consequence of external stress. The discovery of regulated forms of necrotic cell death, such as necroptosis, does not challenge the notion that necrotic demise is caused by relatively nonspecific catastrophic events, such as rapid mitochondrial dysfunction or massive oxidative stress. However, it establishes that these execution events may be mediated by specific and "druggable" sets of discreet biochemical processes, akin to apoptosis, ultimately leading to necrotic morphology. In other words, a subset of necrotic cell death can result from regulated processes actively executed from within the cell in response to extracellular signals, and this sequence of events can be inhibited by small molecules. These findings offer an unprecedented opportunity to selectively target pathological necrotic cell death in an array of diseases. In this review, we describe several emerging classes of necroptosis inhibitors, termed necrostatins.

Necroptosis inhibitors

Since the discovery of necroptosis, we have been seeking to identify and optimize low-molecular-weight molecules capable of inhibiting this process in order to elucidate caspase-independent cell death pathways, their roles in disease pathophysiology and to provide potential lead compounds for therapeutic development. A high-throughput screen (HTS) at Harvard Medical School's Institute of Chemistry and Cellular Biology (ICCB) of > 150,000 compounds in U-937 monocytes induced to undergo necroptosis in the presence of TNF-α and the pan-caspase inhibitor zVAD.fmk has to date revealed over 10 distinct structural classes of necroptosis inhibitors. From among these compounds, four series (exemplified by compounds **1**-**4**; Fig. 1) have been the subject of medicinal chemistry optimization and further cellular biological characterization. In addition, one of these series (*i.e.*, **1**) has also been the subject of more extensive *in vivo* pharmacological studies in various animal disease models.

A fairly extensive structure-activity relationship (SAR) study of the Nec-1 series, starting with **1** identified during the HTS campaign, was conducted (25). For the SAR studies, necroptosis was directly induced in FADD-deficient Jurkat cells utilizing TNF-α. The SAR study revealed that both the indole and hydantoin portions of the molecule were resistant to modifications (Fig. 2). For example, substituents in the 2- or 4-position of the indole ring were not tolerated. In addition, significant reductions in activity were found when substituents were placed in the 1-, 5- or 6-position. However, increases in necroptosis-inhibitory activity were achieved with the introduction of small electron-donating (*i.e.,* Me or OMe) or electron-withdrawing (*i.e.,* Cl) groups to the 7-position of the indole.

For the hydantoin portion of the molecule, the SAR study revealed that both carbonyl groups were necessary. Introduction of a urea in place of the thiourea in **1** did not compromise necroptosis-inhibitory activity, but did result in reduced cytotoxicity at higher compound concentrations and provided greater metabolic stability as assessed in mouse liver microsomes. Small nonbranched alkyl groups (*i.e.*, Me) on the imide nitrogen were best. Hydrogen, large or branched alkyl groups were not tolerated on the imide nitrogen. The amide nitrogen of the hydantoin also did not tolerate substitution or replacement with a methylene. However, replacement with oxygen to give an oxazolidine-2,4-dione was tolerated. Unfortunately, this change resulted in reduced microsomal stability. The stereochemistry present in the hydantoin ring also had an influence on necroptosis-inhibitory activity, with the (*R*)-enantiomer providing greater activity compared to the (*S*)-enantiomer.

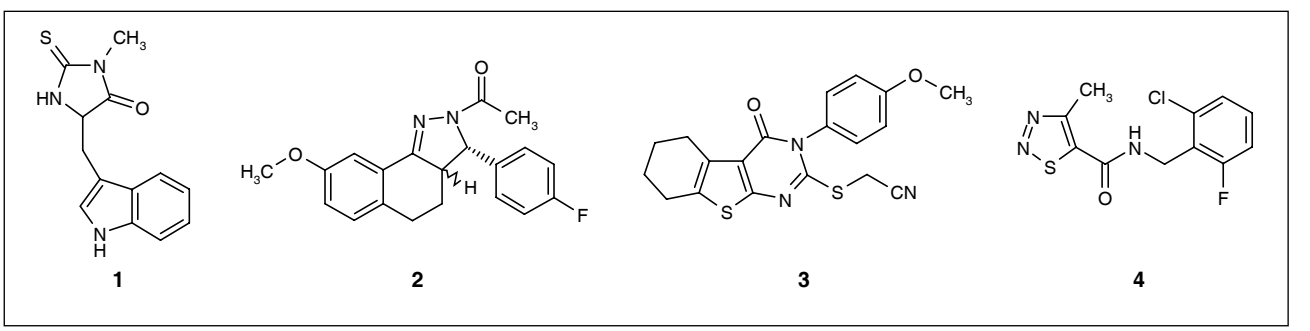

Fig. 1. Necrostatins identified during a high-throughput screening campaign.

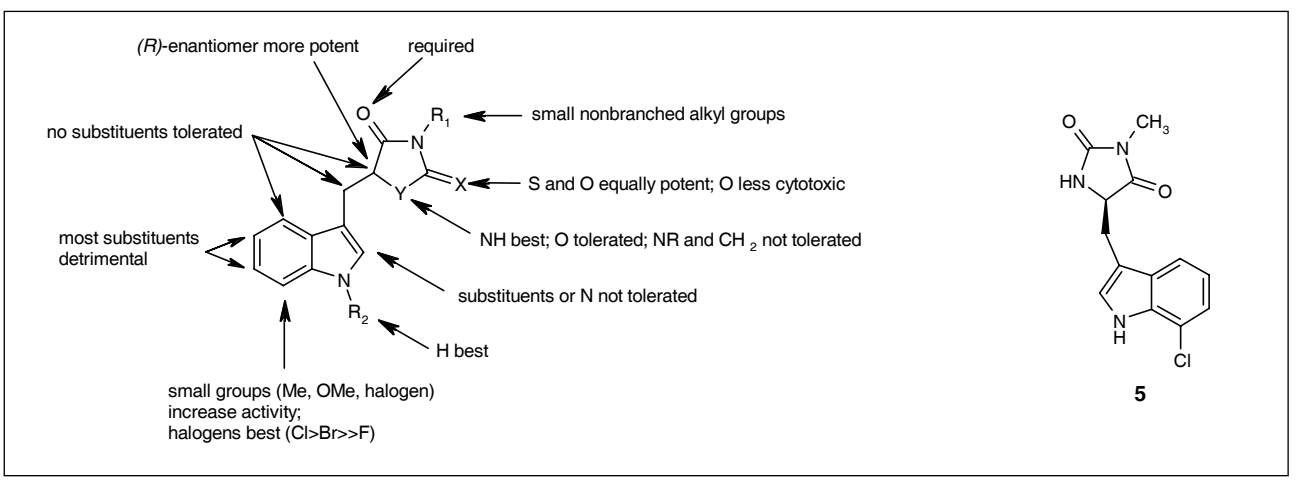

Fig. 2. Summary of Nec-1 series SAR and optimized compound **5**.

Finally, the methylene linker between the indole and hydantoin was also found to be important for activity. Truncation, homologation or addition of substituents to the methylene was detrimental to necroptosis-inhibitory activity. Also, introduction of a double bond between the carbon linker and the hydantoin to give a planar molecule resulted in complete loss of inhibitory activity.

The result of the SAR study was optimized compound **5**, which demonstrated an EC_{50} value of 0.05 μM for protecting FADD-deficient Jurkat cells from TNF-α-induced necroptosis. Compound (±)-**5** exhibited 78.7% protein binding in human plasma, had a $LogD_{7.4}$ of 1.90 and solubility in phosphate buffer (pH 7.4) of 130 μg/ml. This compound demonstrated adequate pharmacokinetic characteristics following i.v. administration of 1 mg/kg to male mice, with an AUC of 16,461 ng.min/ml, a $t_{1/2}$ of 67 min, a volume of distribution (V_{ss}) of 2495 ml/kg (3.4 times the total body water in mouse) and a clearance (CL_s) of 61 ml/min/kg (0.68 times mouse hepatic blood flow). A brain concentration of about 0.8 μM was reached 30 min after administration, although the compound was cleared from both the brain and plasma within 180 min postinjection. The compound also partitioned into the brain with a brain to plasma ratio of 2.4 to 1.

An SAR study of the Nec-3 series (26), starting with **2**, for inhibition of necroptosis in FADD-deficient Jurkat cells utilizing TNF-α to induce cell death revealed that the (3*R*,3a*R*)-rel-isomer (corresponding to the 3- and 3a-hydrogens being in a *syn* orientation) was the significantly more active diastereomer (Fig. 3). Furthermore, the tricyclic ring system was necessary for activity. Removal of the central ring to give a diaryl-substituted dihydropyrazole resulted in loss of necroptosis-inhibitory activity. The benzylic methylene in the central ring could be replaced with sulfur. However, replacement with oxygen resulted in a slight decrease in activity, while replacement with a sulfone gave a significant decrease in activity.

Substituents on the 6-, 7- or 9-position of the fused benzene ring were not tolerated. However, introduction of small electron-donating (*i.e.*, OMe) or electron-withdrawing (*i.e.*, F) groups at the 8-position gave increased activity. Likewise, introduction of small nonbranched electron-donating (*i.e.*, OMe) groups at the 4-position of the pendent phenyl ring also resulted in increased activity.

Finally, the amide at the 2-position of the tricyclic ring was necessary. Reduction to an alkyl amine resulted in complete loss of activity. In addition, replacement of the amide with a sulfonamide, urea or carbamate was not tolerated. Introduction of a hydroxyl or alkoxide group on the α-position of the amide also gave increased activity. The result of the initial SAR study for protecting FADD-deficient Jurkat cells from TNF-α-induced necroptosis was optimized compound **6**, which had an EC_{50} value of 0.29 μM. Evaluations of compounds from the Nec-3 series in liver microsomal stability assays are currently ongoing.

An SAR study of the Nec-5 series (27), starting with **3**, revealed that the cyanomethyl thioether was required for activity (Fig. 4). Although a variety of alkyl substituents were tolerated on the thiophene ring, the derivatives that demonstrated the best activity contained an aryl ring fused to the thiophene. Finally, introduction of electron-donating groups to the 3- and 4-positions of the pendent phenyl ring gave increased potency. The result of the SAR study was optimized compound **7**, which demonstrated an EC_{50} value of 0.15 μM for protecting FADD-deficient Jurkat cells from TNF-α-induced necroptosis. This compound also demonstrated excellent mouse liver microsomal stability ($t_{1/2}$ = 192 min). *In vivo* evaluation of compounds from the Nec-5 series in pharmacokinetic studies and in animal models of disease following acute administration are currently ongoing.

An SAR study of the Nec-4 series (28), starting with **4**, for inhibition of necroptosis in FADD-deficient Jurkat cells

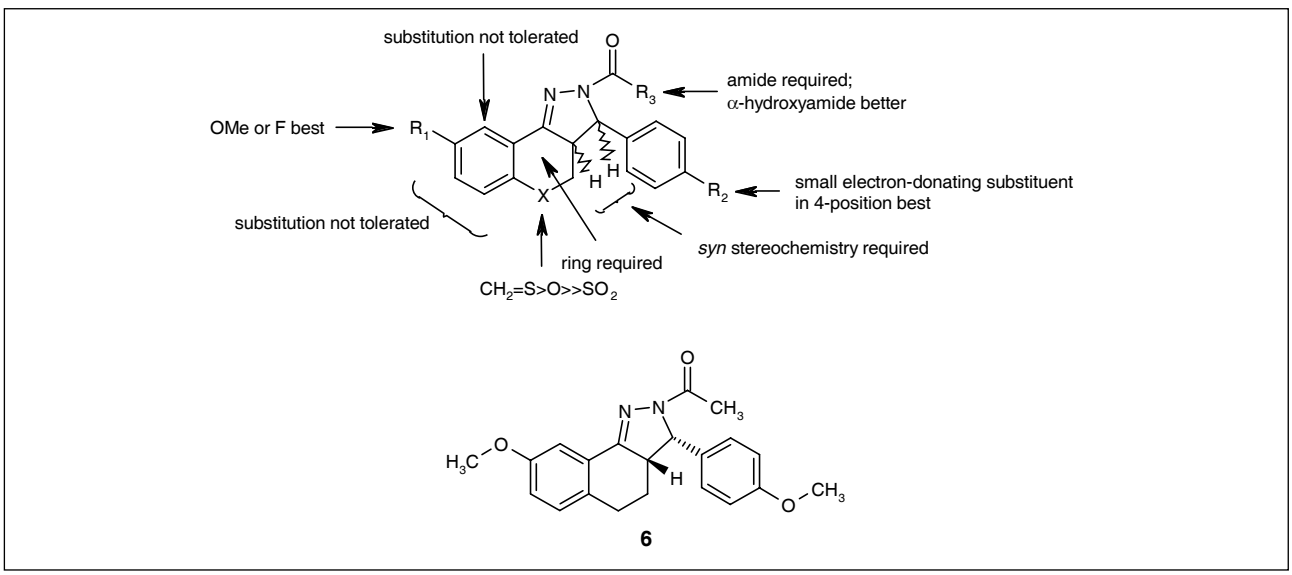

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Fig. 3. Summary of Nec-3 series SAR and optimized compound **6**.

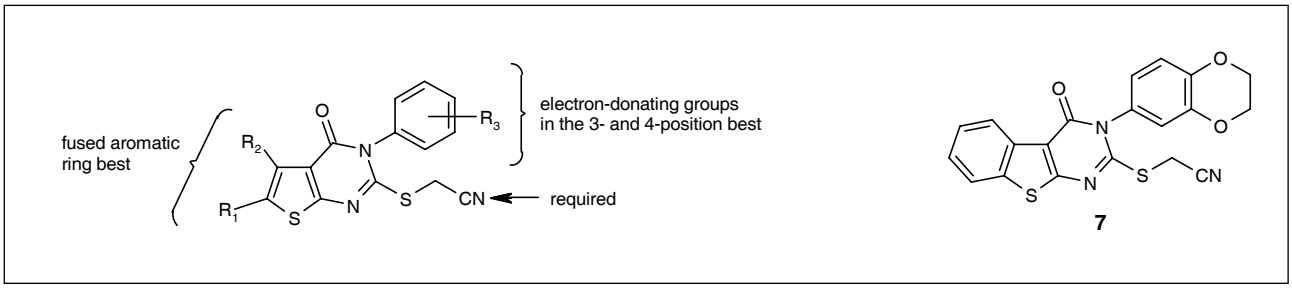

Fig. 4. Summary of Nec-5 series SAR and optimized compound **7**.

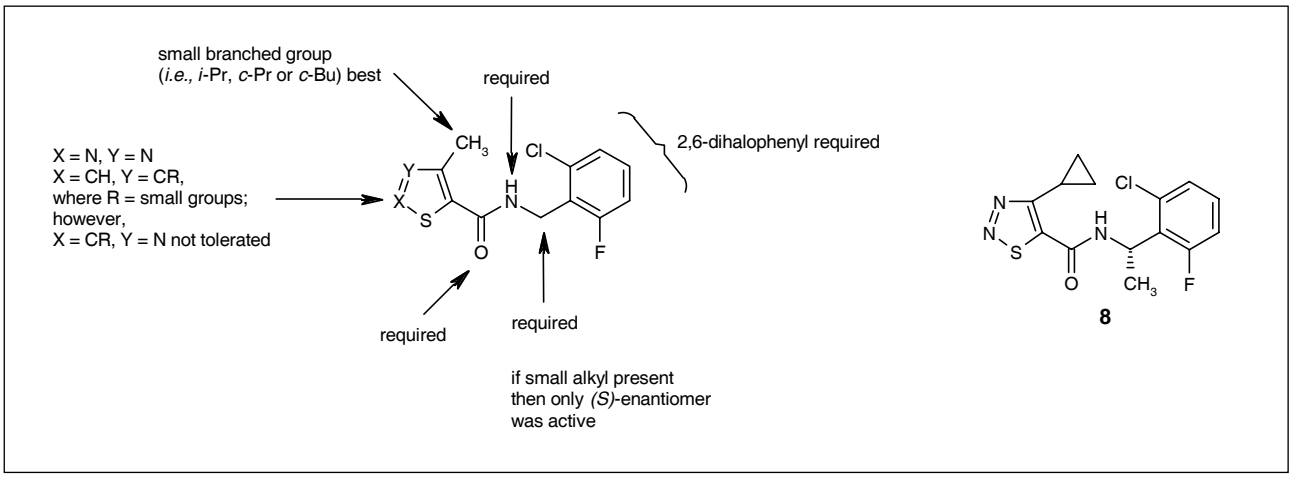

Fig. 5. Summary of Nec-4 series SAR and optimized compound **8**.

utilizing TNF-α to induce cell death revealed that the alkyl group in the 4-position of the [1,2,3]thiadiazole ring was important for activity (Fig. 5). Introduction of small branched or cyclic alkyl groups (*i.e., i*-Pr, *c*-Pr or *c*-Bu) was optimal. However, larger branched (*i.e., t*-Bu) or cyclic (*i.e., c*-Hex) alkyl groups or phenyl were detrimental to activity. In addition, the secondary amide was necessary and the benzylic group was also important for activity. Truncation to an anilide or homologation to a phenethyl resulted in loss of necroptosis-inhibitory activity. Furthermore, small alkyl groups (*i.e.*, Me) were tolerated on the benzylic carbon. However, when such a group was present, all the necroptosis-inhibitory activity resided with the (*S*)-enantiomer. The 2,6-dihalogen arrangement on the benzyl ring was also necessary for activity, and in many cases the 2-Cl, 6-F substitution was optimal. Finally, various replacements of the [1,2,3]thiadiazole heterocycle were examined. Replacements with thiazoles or oxazoles were detrimental. However, necroptosis-inhibitory activity could be retained by replacement with a thiophene, although with a slight decrease in activity. The result of the SAR study was optimized compound **8**, which demonstrated an EC_{50} value of 0.28 μM for protecting FADD-deficient Jurkat cells from TNF-α-induced necroptosis. Evaluations of compounds from the Nec-4 series in liver microsomal stability assays are currently ongoing.

Potential clinical indications for necroptosis inhibitors

Necroptosis inhibitors may offer effective treatment for an array of acute and chronic human diseases where necrosis plays a significant role. Several indications that might benefit from necrostatin therapy include cerebral ischemia, traumatic brain injury, myocardial infarction, retinal ischemia, liver injury, chemo/radiation therapy-induced necrosis, acute necrotizing pancreatitis and possibly some neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS).

Cerebral ischemia is a condition where parts of the brain do not receive adequate blood flow, most commonly due to a cerebral arterial obstruction. This condition deprives the tissue near the obstruction (the ischemic core) of vital supplies of oxygen and nutrients, resulting in the immediate initiation of multiple biochemical events that lead to cell death (29, 30). Typically, cell death in the ischemic core occurs very rapidly (minutes to hours) and is necrotic. However, over time (hours to days) the zone of cell death expands to surrounding tissue (ischemic penumbra) and is morphologically characterized by both necrotic and apoptotic cell death.

Cerebral ischemia is the most common form of stroke (accounting for > 85% of cases) and is arguably one of the most prevalent conditions for which adequate thera-

pies are lacking. The only approved drug for cerebral ischemia is recombinant tissue plasminogen activator (t-PA), which is used to restore adequate blood flow to the affected brain regions. However, t-PA must be administered only to patients experiencing an ischemic stroke, not a hemorrhagic stroke, and within 3 h of the ischemic event, which is difficult to accomplish in practice due to the fact that most stroke patients do not present for treatment within this narrow time window. The scarcity of available therapeutic options for cerebral ischemia is not due to a lack of effort. However, a staggering number of potential neuroprotective drug candidates have failed clinical trials over the past several decades, primarily due to lack of efficacy (31).

Since cerebral ischemia is characterized by necrotic cell death in both the ischemic core and the penumbra, necrostatin therapy may be beneficial. Necroptosis inhibitors from the Nec-1 series, but not inactive analogues, were able to statistically significantly reduce infarct volume in the mouse middle cerebral artery occlusion (MCAO) model of cerebral ischemia upon administration 5 min preocclusion or 6 h postocclusion (19). Interestingly, the reduction in infarct volume was additive when a necroptosis inhibitor was given in combination with the pan-caspase inhibitor zVAD.fmk. This result further illustrates that multiple cell death mechanisms are operable during MCAO in animals. This may also be the case in humans.

Traumatic brain injury. Although early clinical studies failed to demonstrate cerebral ischemia following traumatic brain injury, more recent work has shown reduction in focal or global cerebral blood flow within hours of the initial insult. In addition, the onset of ischemia was proposed to influence brain viability and patient outcome following brain trauma. In many cases, necrosis is induced immediately following traumatic brain injury. In addition, apoptosis can follow and continue to occur months after the initial insult (32).

In a mouse model of controlled cortical impact (CCI), necroptosis inhibitors from the Nec-1 series, but not inactive analogues, administered prior to CCI statistically significantly decreased cells with plasmalemma permeability in injured cortex and dentate gyrus regions at 6 h, improved motor performance at 1-7 days and performance in the Morris water maze memory paradigm at 8-14 days, and decreased brain tissue damage at 2 and 5 weeks post-CCI (33). Beneficial effects of the necroptosis inhibitors were also observed when administered 15 min, but not 30 min, post-CCI. The beneficial time window in the CCI model was notably shorter than in the MCAO cerebral ischemia model and may reflect different induction periods for necroptosis in the two brain injury models. Consistent with this idea, plasmalemma permeability in cortical and hippocampal neurons peaks at 1 h after CCI but occurs several hours after reperfusion in focal stroke (34, 35). The additional finding that Nec-1 reduced acute cellular plasmalemma permeability but did not reduce caspase-3-positive cells in either the dentate gyrus or cortex regions is consistent with its specificity for non-apoptotic cell death. Interestingly, a novel antiinflammatory effect of the necroptosis inhibitors was noted during these studies, with reduced brain neutrophil influx and a striking decrease in microglial activation observed at 48 h post-CCI. Therefore, these data suggest that regulated necrosis is part of the cell death continuum following brain trauma, responsible for a significant portion of the resulting brain damage, and that necroptosis pathways may be linked to mechanisms regulating motor and cognitive outcome after traumatic brain injury.

Myocardial infarction is in many ways fundamentally similar to cerebral ischemia, except that it occurs in the heart. It usually arises from blockage of a coronary artery following disruption of an atherosclerotic plaque. Once the blockage occurs, a series of pathophysiological events begins, culminating in myocardial cell lysis beginning about 5 h postocclusion. The myocardial necrosis is also associated with the release of various cardiac enzymes (*e.g.,* creatine kinases) and proteins (*e.g.*, TNF-α) into the bloodstream (36).

Smith *et al.* (37) have shown that a necroptosis inhibitor reduced peroxide-induced cell death in C_2C_{12} and H9c2 myocytes. In addition, the inhibitor reduced infarct size in isolated perfused mouse hearts (Langendorff model). An inactive necroptosis inhibitor also demonstrated activity, albeit providing less protection. A necroptosis inhibitor also reduced infarct volume in mice following left anterior descending coronary artery ligation. In this experiment, the inactive necroptosis inhibitor lacked efficacy. Collectively, these results demonstrate that necroptosis appears to be operative during myocardial ischemia/reperfusion injury in rodents and that necroptosis inhibitors provide protection. Furthermore, recent genetic analysis using cyclophilin D-deficient mice (38) demonstrated that Nec-1 affects the same pathway of cell death that was previously attributed to unregulated myocardial necrosis (39, 40), providing the first direct demonstration that a significant portion of pathological necrosis *in vivo* results from an RIP1 kinase-dependent regulated cellular pathway.

Retinal ischemia. This ocular condition shares many of the common elements of cerebral ischemia since the retina is an extension of the CNS, including neuronal depolarization, Ca^{2+} influx, oxidative stress and increased glutamatergic stimulation (41). Given the similarities of this condition to both cerebral ischemia and myocardial infarction, necroptotic cell death may also be involved. Pharmacological evaluation of necrostatins in animal models of retinal ischemia is needed to support the potential use of necroptosis inhibitors for treating this ocular condition.

Liver injury. Liver cell death can be induced by an array of initiators, including hypoxia, toxins and TNF-α. Similar to other tissues and organ systems, the resulting cell death can be apoptotic or necrotic (42, 43). Acute viral hepatitis is one condition that can result in significant liver necrosis. Subacute hepatitis is a serious and often fatal condition where extensive hepatocellular necrosis develops over the course of several weeks to months

(44). Another important cause of liver necrosis is chemically induced hepatic injury following exposure to a variety of toxins, including natural products (*e.g.,* aflatoxins and pyrrolizidine alkaloids), metals and metal salts (*e.g.,* $CuSO_4$, beryllium and selenium) and industrial/synthetic compounds (*e.g.,* chloroform and tannic acid) (45). This latter category also includes therapeutic compounds such as acetaminophen (46) and the immunosuppressant drug FK-506 (tacrolimus) (47), which at high doses can result in potentially fatal hepatic necrosis.

Cancer chemo/radiation therapy-induced necrosis. Organ and tissue necrosis can result from both chemotherapy and radiation therapy for the treatment of neoplasms. For example, cerebral radiation necrosis is a significant complication following radiation treatment of brain cancers (48). The chemotherapeutic agent cisplatin, which is an effective treatment for an array of malignancies, leads to reduced renal function in 25-35% of patients after a single administration, resulting from both necrosis and apoptosis of renal epithelial cells. This chemotherapeutically induced acute renal failure was proposed to involve upregulation of the TNF receptor TNFR2 (49). In addition, a combination of chemotherapy and radiation therapy for some head and neck tumors can result in laryngeal necrosis as a significant side effect (50). Conceivably, prophylactic treatment with necrostatins prior to chemo- and/or radiation therapy might provide an effective means to minimize the necrotic cell death associated with these anticancer therapies. The feasibility of such a strategy will depend on the relative contribution of necrotic death to normal tissue injury *versus* the elimination of cancer cells, which has yet to be explored.

Acute necrotizing pancreatitis. The etiology of acute necrotizing pancreatitis is unclear. Several potential causes include permanent or transient obstruction of the common bile duct or the main pancreatic duct (*i.e.,* the Wirsung duct), fibrosis of the sphincter of Oddi at the surface of the duodenum or a neoplasm in these areas. In addition to obstructions, spasms of the sphincter of Oddi are also a potential cause. Acute necrotizing pancreatitis is commonly associated with biliary tract disease, alcoholism, trauma (including from abdominal surgery) and several types of infections, including toxoplasmosis and adenovirus infection (51).

One or more of these insults to the pancreas can initiate a cascade of events leading to pancreatic enzyme activation that ultimately results in gross pathological changes to the pancreas, potentially including hemorrhage and necrotic pancreatic tissue (52). In severe cases, these processes can spread to neighboring tissues, including the colon. Acute necrotizing pancreatitis has a mortality rate of about 20% (53) and this rate increases to near 50% when hemorrhagic and necrotic tissue is present. Although the specific role of necroptosis in acute pancreatitis has not yet been established, a recent study suggested a role for RIP1 kinase in necrotic, but not apoptotic, pancreatic injury (54). These findings suggest that necrostatins may hold promise in reducing mortality from the acute form of this disease.

Neurodegeneration. In many sporadic and inherited neurodegenerative diseases, such as Alzheimer's, Huntington's and Parkinson's diseases, multiple sclerosis and ALS, markers of apoptosis are present. In addition, apoptosis is also present in many of the currently available animal models of these diseases, suggesting that it is likely contributing to the observed cell death in these diseases (55). Although necrosis is definitely present and in some cases the predominant form of cell death in acute neurodegeneration (*i.e.,* cerebral ischemia), its role in chronic neurodegenerative diseases is less clear. Its potential role, particularly at the early stages of these diseases, is further complicated by the slow disease progression in humans and by the lack of adequate animal models that recapitulate the time course and conditions of these diseases (56). Therefore, the role of regulated caspase-independent forms of necrosis, such as necroptosis, in these diseases is currently unknown. Perhaps with the advent of necroptosis-specific biomarkers a determination of the role of necroptosis in neurodegenerative diseases can be defined. If this form of cell death is indeed operative during the course of the disease, particularly during the early phase, then necrostatins may offer an opportunity for disease-modifying therapy.

Conclusions

The emergence of regulated caspase-independent cell death pathways with morphological features of necrosis presents new opportunities to design therapeutic strategies for treating diseases that manifest with a significant necrotic component. One of these pathways, necroptosis, has been shown to be induced directly following death receptor stimulation in an array of cell types and by various stimuli, establishing that necrotic demise can result from controlled cellular processes. In addition, this cell death pathway can be inhibited by small molecules that possess desirable pharmaceutical properties (*i.e.*, solubility, metabolic stability, tissue distribution, etc.) and these inhibitors have been used to demonstrate that necroptosis is operative in a host of animal disease models. Furthermore, these results raise the possibility that this cell death pathway may play a role in human diseases, such as cerebral ischemia, traumatic brain injury and myocardial infarction, as well. With a more complete understanding of necroptosis regulation and further development of necrostatin molecules, the treatment of many currently incurable human necrotic diseases may ultimately become possible.

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