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A Review of Multi-Threat Medical Countermeasures against Chemical Warfare and Terrorism

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The Multi-Threat Medical Countermeasure (MTMC) hypothesis has been proposed with the aim of developing a single countermeasure drug with efficacy against different pathologies caused by multiple classes of chemical warfare agents. Although sites and mechanisms of action and the pathologies caused by different chemical insults vary, common biochemical signaling pathways, molecular mediators, and cellular processes provide targets for MTMC drugs. This article will review the MTMC hypothesis for blister and nerve agents and will expand the scope of the concept to include other chemicals as well as briefly consider biological agents. The article will also consider how common biochemical signaling pathways, molecular mediators, and cellular processes that contribute to clinical pathologies and syndromes may relate to the toxicity of threat agents. Discovery of MTMC provides the opportunity for the integration of diverse researchers and clinicians, and for the exploitation of cutting-edge technologies and drug discovery. The broad-spectrum nature of MTMC can augment military and civil defense to combat chemical warfare and chemical terrorism.

Introduction

The list of military chemical threat agents has lengthened over the last century. World War I saw the development and deployment of edemagenic and choking agents such as chlorine, phosgene, and cyanide, and blister gases such as sulfur mustard (SM).^{1,3} World War II cultivated Germany's development of nerve gases such as tabun, sarin, and soman,³⁻⁶ and this class of agent was used in the 1980 to 1988 Iraq-Iran conflict^{7,8} and in the 1994 to 1995 terrorist attacks in Japan.⁹⁻¹¹ The Cold War yielded more lethality refinements in nerve agents to include development of VX.¹² Consistent with past experience, new threat agents with novel mechanisms of toxicity may evolve.

Countermeasures for defending deployed military personnel against weaponized chemical warfare agents do not seamlessly transition to military force protection and civil defense against chemical terrorism. Terrorist threat agents include all classes of chemical warfare agents, toxins, and a broader menu of toxic industrial chemicals. The large number of threats and potential for use of multiple toxic substances in combination can complicate agent identification and medical response. A terrorist assault might more closely resemble the chemical accident at Bhopal, India, than a chemical warfare attack. Symptoms may

be closer to smoke inhalation and acute respiratory distress syndrome (ARDS) than to the cholinergic crisis caused by nerve agent poisoning. Research into medical countermeasures against weaponized battlefield chemical warfare agents has focused on distinct classes of nerve, blister, choking, or edemagenic agents and pathologies specific to these agents, e.g., nerve agent-induced seizure, phosgene-induced ARDS, and SM blistering. The present challenge of defending against the myriad of threat chemicals and toxins necessitates a more collective and comprehensive strategy that more generally addresses the symptoms and pathology of chemical toxicity rather than a specific chemical mechanism.

The Multi-Threat Medical Countermeasure (MTMC) hypothesis for chemical toxicity is analogous to the broad-spectrum approach used for protecting against infectious agents authored by Dr. Ken Alibek, former Deputy Chief of Biopreparat, the civilian arm of the former Soviet Union's biological weapons program.^{13,14} It may be possible to exploit the fact that the diverse chemistry, sites, and mechanisms of action of toxic chemicals often involve common underlying biochemical signal pathways. These common pathways are key in initiating the cellular processes that contribute to clinical pathologies caused by chemical exposures. Clinically defined syndromes or symptoms that represent pathologies of respiratory, neuronal, cardiac, cutaneous, or other systems to include anaphylactic shock, bronchospasm, ARDS, seizure, excitotoxin-like neuronal degeneration, and blistering are observed after exposure to chemical warfare agents, hazardous chemicals, toxins, and even infectious agents. The MTMC hypothesis suggests that common biochemical pathways contribute to the toxicity that causes the pathologies associated with different classes of toxic chemicals. MTMC drugs that target these key pathways might create a physiological "quiescent" state that protects against various chemical insults.¹⁴

MTMC and Inflammatory Response

The fact that inflammation causes or contributes to numerous pathologies and that many toxic chemicals provoke an inflammatory response is well established (for review, see Refs. 14-16). Although evidence for a significant role for inflammation in chemical toxicity continues to accumulate, inflammatory response is still often regarded as merely collateral to chemical insult and secondary to toxicity.¹⁴⁻¹⁶ The evolution over the last decade of the concept that inflammatory response is a key element of chemical toxicity that allows for the development of anti-inflammatory drugs as multi-agent countermeasures is unique to the MTMC hypothesis.¹⁴⁻¹⁶ The MTMC hypothesis encompasses the interaction of multiple toxic agents and diverse countermeasure drugs on mediators and signaling pathways that cause or sustain inflammation. Whether inflamma-

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tion is a collateral biomarker or causative contributor to chemical toxicity is controversial. However, in either case inflammation can provide a common denominator to facilitate deciphering the relationships between agents, drugs, signaling pathways, and cellular processes pertinent to predicting candidate MTMC drugs.¹⁴

A major tenet of the pharmacology of anti-inflammatory drugs is that drug action can result from the specific inhibition of molecular mediators of inflammation or from modulation of the underlying biochemistry that initiates or sustains an inflammatory response. Different inflammatory mediators, pathways, and cell populations can be induced or recruited by different conditions or insults. This multifaceted nature of inflammation may require the use of numerous pharmacologically distinct anti-inflammatory drugs to contend with the different manifestations of inflammatory pathology. However, enough uniformity of biochemical pathways of inflammation exists to allow for development of broad-spectrum anti-inflammatory drugs.

The biochemical pathways associated with chemical toxicity can involve proteases, inflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-8, and other molecules such as platelet activating factor (PAF), *N*-methyl-D-aspartate (NMDA) glutamate receptors, acetylcholine (ACh), substance P, and poly(ADP-ribose) polymerase (PARP) (for review, see Ref. 14). These mediators and receptors can influence inflammatory responses associated with cellular processes such as degranulation, apoptosis, and necrosis that contribute to pathologies caused by chemical agents. Therefore, many classes of compounds used as countermeasures to chemical warfare agents such as PARP inhibitors, proteases inhibitors, adenosine agonists, and NMDA receptor antagonist, although not chiefly thought of as anti-inflammatory drugs, have anti-inflammatory pharmacology (Table I) (for review, see Ref. 14).

The diverse biochemical pathways that influence inflammatory responses may explain why many pathologies associated with intoxication by chemical agents have an inflammatory component. Along this line of reasoning, it is not surprising that the majority of countermeasure drugs that have shown efficacy against edemagenic, vesicant, and nerve agents have anti-inflammatory pharmacology (for review, see Ref. 14). The presence of inflammatory responses and anti-inflammatory actions of

countermeasures implicates inflammation as a cause or a resulting biomarker of the toxicity of these agents. The extent to which common inflammatory mechanisms are coupled to chemical agent toxicity will determine the feasibility of developing anti-inflammatory MTMC.

Inflammation Associated with Edemagenic, Blister, and Nerve Agents

Inflammatory response induced by the prototypic edemagenic agent phosgene is the primary cause of the frequently lethal ARDS.^{17,18} ARDS is also associated with pulmonary toxicity of the acetylcholinesterase-inhibiting organophosphorus insecticide thionazine,¹⁹ SM,¹⁴ and other toxic chemicals, as well as toxins, infectious-agent pneumonia, shock, sepsis, burns, and other trauma (Table II). SM dermal-epidermal separation is similar to that caused by proteolysis and certain bullous diseases, and this has fostered the hypotheses that SM vesication involves proteolytic and/or inflammatory responses.¹⁵⁻²⁰ Experimental evidence has accumulated over the last decade demonstrating SM-increased proteases and the expression of inflammatory enzymes, gene products, mediators, and receptors in tissue or cell cultures.^{14,15,19-23} Inflammatory enzymes and cytokines have also been reported in the skin of SM-exposed animals.^{24,25}

Cholinergic crisis is the paramount toxicological event in nerve agent intoxication, causing acute toxicity and precipitating seizure and neuronal degeneration.²⁶ However, along with cholinergic crisis anaphylactoid reactions, pathological proteolytic activity and inflammatory cytokines have also been reported in nerve agent-intoxicated animals.^{14,27-31} The initial observations of anaphylactoid reactions associated with soman poisoning reported by Doeblner et al.²⁸ and Newball et al.²⁹ nearly a quarter century ago have recently again been considered by Gilat et al.³⁰ in sarin-intoxicated animals. Anaphylactoid reactions, by definition, occur upon initial exposure to a chemical independent of antibody production. Anaphylactoid reactions and nerve agent intoxication can generate acute symptoms such as convulsions, bronchoconstriction, respiratory failure, circulatory collapse, and death within a few minutes.^{14,16} Thus, anaphylactoid reactions,^{14,16} like the more extensively investigated cholinergic crisis and excitotoxin-like neuronal degeneration, are a clinically well-defined and potentially lethal syndrome associated with nerve agent intoxication.

McLeod³² noted neuronal and neurophil degeneration and necrosis associated with soman-induced brain lesions. The pattern of injury was similar to that caused by epilepsy and isch-

TABLE I
PUTATIVE MEDIATORS OF INFLAMMATION AND POTENTIAL
TARGETS FOR MTMC DRUGS

Mediator (Site of Action)	Candidate MTMC Drug
Proteases [proteolysis, proteases receptors (α)]	Protease inhibitors
Inflammatory cytokines	Soluble cytokine receptors
PAF (PAF α)	PAF α antagonist (benzodiazepines)
Glutamate (NMDA α)	NMDA α antagonist
Acetylcholine (acetylcholine α)	Anticholinergics (atropine)
PARP	PARP inhibitors

Other possible MTMC drugs with more general sites of anti-inflammatory action include adenosine agonists, corticosteroids, and nonsteroidal anti-inflammatory drugs. Of particular interest are drugs that have efficacy against SM toxicity in the MEVM and also inhibit SM-increased IL-8 in HEK cultures (for review, see Ref. 14).

TABLE II
CHEMICAL, TOXIN, AND INFECTIOUS AGENTS AND INSULTS THAT
CAUSE ARDS

Agent/Insult	Classification
Organophosphate compounds	Anticholinesterase
Sulfur mustard	Blister
Phosgene	Inhalation edemagenic
Mycotoxin	Biotoxin
Anthrax, smallpox, Ebola, and severe acute respiratory syndrome	Infectious agents
Pneumonia, shock, sepsis trauma, and burns	Acute lung/organ dysfunction

emic brain injury. Some animals also had cardiac lesions characterized by acute necrosis with subsequent mild inflammation and fibrosis. Inflammatory cytokines such as TNF, IL-1, and IL-8 are associated with recruitment of inflammatory cells and cellular processes such as degranulation, apoptosis, and necrosis. Inflammatory cytokines are causative factors in phosgene-associated ARDS, they contribute to SM injury, and they are implicated in nerve agent seizure and neuronal degeneration.^{14,17,18,24,25,31,32} Skin from mice SM exposure sites and the brains of soman-intoxicated rats demonstrate increased IL-1 β mRNA.^{22,23,31} IL-1 is an inflammatory cytokine that can precipitate other cytokines such as TNF, IL-6, and IL-8, and such a cytokine cascade is implicated in SM toxicity.²² Svensson et al.³¹ have demonstrated that soman induces the inflammatory cytokine IL-1 β in rat brain, and this activity is highly correlated with convulsions. Williams et al.³³ confirmed IL-1 β , and further observed an acute and transient upregulation of other inflammatory gene response, i.e., TNF, IL-6, E-selectin, and intercellular adhesion molecule-1 caused by exposure to soman. The authors suggest that these molecules may be involved in soman-induced brain injury. Eriksson et al.³⁴ demonstrated that NMDA antagonists could suppress excitotoxin-induced IL-1 β mRNA in the rat brain. Furthermore, Vezzani et al.³⁵ have protected mice against NMDA receptor-dependent seizures with soluble IL-1 receptors that specifically antagonize IL-1 activity. The authors suggest that the interaction between IL-1 and IL-1 receptors represents a crucial mechanism of seizure and a target for development of anticonvulsant drugs. The role of inflammatory cytokines in edemagenic, nerve, and blister agent toxicity supports cytokine antagonists as candidate MTMC.

Anti-Inflammatory Action of Medical Countermeasures to Edemagenic, Blister, and Nerve Agents

Anti-inflammatory drugs such as ibuprofen are the drugs of choice in reducing phosgene toxicity.^{17,18} The majority of drugs that have shown efficacy against SM in animal models have anti-inflammatory pharmacology (for review, see Ref. 14). Our institute has screened more than 400 compounds in the mouse ear vesicant model (MEVM) for cutaneous injury; 19 compounds reduced SM histopathology greater than 50%.³⁶⁻³⁸ The 19 compounds include seven listed as anti-inflammatory drugs, consisting of five capsaicin analogs, a single cyclooxygenase inhibitor, indomethacin, and a calmodulin antagonist, fluphenazine.³⁶⁻³⁸ The three protease inhibitors and three PARP inhibitors included in the list of antivesicant drugs also have the potential to inhibit inflammatory responses.^{14,39} The remaining six anti-vesicant drugs, sodium 3-sulfonatopropyl glutathionyl disulfide, 3% hydrogen peroxide gel, dimercaprol, and three other mercaptopyridines analogs are listed as SM scavengers.^{36,38} However, mercaptopyridine-like compounds have demonstrated anti-inflammatory pharmacology to include inhibition of the cellular release of the inflammatory cytokine IL-1.⁴⁰ Therefore, 17 of the 19 compounds with efficacy against SM toxicity have anti-inflammatory pharmacology, the exceptions being two of the listed SM scavengers. Furthermore, antivesicant drugs with anti-inflammatory pharmacology generally demonstrate inhibition of HD-increased IL-8 in human epidermal keratinocyte

(HEK) cultures (Ref. 41; FM Cowan, CA Broomfield, and WJ Smith, unpublished observations). Collectively, the MEVM and in vitro SM-increased IL-8 HEK assays effectively screen, not just for vesicant countermeasures, but also for anti-inflammatory drugs that inhibit IL-8. These models further identified drugs such as capsaicin analogs as better antivesicants and anticytokine drugs than major classes of currently available corticosteroids and nonsteroidal anti-inflammatory drugs.

The anti-inflammatory action of nerve agent countermeasures to include atropine, carbamates, and benzodiazepines has been extensively reviewed.^{14,16,42} For example, a role for synergy between mediators of anaphylactic response and cholinergic status "hypersensitivity" is accepted for asthma-related lethal bronchoconstriction, and has been implicated for nerve agent-induced lethal bronchoconstriction.^{14,16,43,44} Kotev⁴⁴ has demonstrated that soman-induced bronchial spasm was greatly intensified not only by ACh, but also by histamine. Hypersensitivity to inhalation of the cholinergic compounds such as methylcholine or the autacoid histamine is a diagnostic test for asthma, and anticholinergics are sometimes used as supplementary treatment.⁴⁵

Inflammatory, neurological, and tissue pathology pathways involve proteolytic processes.¹⁴ Synthesized mediators such as PAF and serine proteases such as tryptase and tissue plasminogen activator (t-PA) in addition to autacoids such as histamine are significant factors in inflammatory and anaphylactic responses.^{14,16,46} Carbamates can inhibit serine proteases that mediate anaphylaxis and prevent anaphylactic response.⁴⁷ The benzodiazepine diazepam is Food and Drug Administration approved for the treatment of seizures associated with nerve agent poisoning.⁴⁸ Benzodiazepines can, in addition to acting as γ -aminobutyric acid receptor agonists, also inhibit PAF receptors.⁴⁹ PAF is a dual proinflammatory and neuromessenger phospholipid molecule that participates in pathological phenomena that include protease synthesis, lethal anaphylaxis, vesication and NMDA glutamate receptor excitotoxicity, seizure, and neuronal degeneration (for review, see Ref. 14). Mice are protected from lethal anaphylaxis by serine protease inhibitors or PAF antagonists.^{46,49} The γ -aminobutyric acid receptor agonist and PAF receptor antagonist actions of benzodiazepines might synergistically protect against nerve agent cholinergic and inflammatory toxicities. Furthermore, proteolytic and inflammatory pathways are also strongly implicated in NMDA receptor-mediated seizure and neuronal degeneration. Knock-out mice deficient in the protease t-PA were resistant to seizure and neuronal pathology caused by excitotoxins (for review, see Ref. 50). Furthermore, the synthetic protease inhibitor tPA-STOP (American Diagnostica, Greenwich, CT) suppressed excitotoxin-induced seizure and neuronal degeneration (for review, see Ref. 50).

Experimental Evidence for Anti-Inflammatory MTMC Drugs

Experimental evidence generated in in vitro and in vivo models of chemical agent toxicity supports the concept of anti-inflammatory MTMC drugs. SM exposure increases the inflammatory IL-8 in HEK and human small airway cell (HSAC) cultures.^{40,51} SM-increased IL-8 in HEK cultures is a biomarker

of dermal inflammatory pathology that is used as an *in vitro* drug screening and treatment model to complement *in vivo* studies in the MEVM.⁴⁰ Likewise, SM-increased IL-8 in HSAC has recently been developed as an *in vitro* model for treatment of inhalation toxicity.⁵¹ HSAC cultures exposed to 25 to 200 μ M SM or 0.2 to 6.4 ppm/min phosgene demonstrated significantly increased IL-8.⁵² A maximum increase of approximately 1,000 pg/mL IL-8 in HSAC cultures exposed to phosgene or SM was observed.⁵² The pattern of IL-8 response, increase to maximum levels followed by inhibition at higher cytotoxic doses, was similar for both agents. Ibuprofen (62, 125, 250, 500, and 1,000 μ M) significantly diminished phosgene-increased IL-8 in SAC cultures exposed to 2 ppm/min phosgene.⁵² Furthermore, the doses of ibuprofen that decreased phosgene-increased IL-8 approximately 50% in HSAC (125 and 250 μ M) also inhibited SM-increased IL-8 in HEK cultures to about the same extent.^{51,53}

Serine protease inhibitors and PARP inhibitors can have anti-inflammatory pharmacology, and members of these two distinct classes of compounds have shown efficacy for nerve and blister agents *in vivo*.^{14,36,46,53} Serine protease inhibitors can prolong the survival of animals intoxicated with the nerve agent soman,⁴¹ and can also protect against vesication caused by the blister agent SM.³⁶ PARP inhibitors can reduce soman-induced neuronal degeneration⁵³ and SM-induced epidermal necrosis.³⁶

Future MTMC Drugs

Candidate anti-inflammatory MTMC drugs to include protease inhibitors, PAF antagonists, PARP inhibitors, NMDA receptor antagonist, and adenosine agonists have been suggested (Table I).¹⁴ Adenosine receptor agonists illustrate the potential and the complexity of MTMC drugs. Van Helden et al.⁵⁴ used the adenosine A1 receptor agonist N6-cyclopentyl adenosine as a countermeasure to soman poisoning. Without any supportive treatment with atropine, oxime, or diazepam, N6-cyclopentyl adenosine protected rats from convulsive activity and respiratory distress and improved 24-hour survival.⁵⁴ Reduced ACh levels in rat brains were reported in this study, and the protection was attributed to cholinergic mechanisms wherein the adenosine A1 receptor agonists binding to the A1 adenosine receptor caused inhibition of ACh release.⁵⁴ However, the cholinesterase-inhibiting nerve agents sarin, tabun, and soman can interact directly with brain A1 adenosine receptors and may competitively alter the action of these agents at adenosine receptor sites.^{14,55}

Adenosine receptors can moderate the activation of other receptors that influence neurotransmitter release and/or synaptic transmission, e.g., ACh and NMDA receptors.^{54,56} A2, A3, and A1 adenosine receptor agonists can further influence mast cell degranulation, and adenosine-directed treatment modalities have been suggested for asthma.⁵⁷ Neutrophil infiltration of dermal inflammatory sites, similar to that also observed in SM pathogenesis, was inhibited by administration of adenosine A1 receptor agonists.^{14,58} This anti-inflammatory action of adenosine agonists was reversed by an NMDA agonist and was mimicked by a glutamate NMDA receptor antagonist.⁵⁸ Hence, in this model, central NMDA receptor activity can control peripheral neutrophil accumulation, and adenosine A1 receptor agonists can influence this NMDA receptor excitation-mediated inflam-

mation.⁵⁸ Virag and Szabo⁵⁹ have further demonstrated that adenosine can inhibit the activation of PARP, and they propose that this may affect cell death and inflammation. Thus, adenosine agonists can influence multiple inflammatory biochemical pathways associated with nerve and blister agent toxicity and are candidate MTMC (Table III).

MTMC Hypothesis, Biological Agents, and Inflammatory Synergy

The activation of inflammatory responses that contribute to pathology is not restricted to chemical agents. T-2 toxin, as a major trichothecene mycotoxin, has some radiomimetic properties, and the results of its action, like the blistering induced by SM, can be alleviated by treatment with glucocorticoid hormones.^{60,61} Moreover, PAF seems to have an important role in the pathophysiology of T-2 toxicosis because its selective antagonist BN 520121 can prolong survival of rats exposed to lethal doses of this toxin.⁶² Trichothecene mycotoxin also induce mesenteric⁶³ and subcutaneous mast cell degranulation⁶⁴ and increased secretion of interleukins IL-1 β and IL-6 *in vitro*. This suggests that superinduction of cytokines might be one of the mechanisms of T-2 toxin-induced tissue damage.⁶⁵ The inflammatory properties of mycotoxin may further contribute to core pathologies such as ARDS. Likewise, inflammatory cytokines and the complication of ARDS are associated with the pathology of infectious agents such as anthrax, smallpox, Ebola, and, of course, the corona virus infection that causes severe acute respiratory syndrome.⁶⁶⁻⁶⁸

The MTMC hypothesis suggests that inflammation is not just a biomarker, but is also a major cause of toxicity for many chemical warfare agents and toxic chemicals. As a counterpoint to the anti-inflammatory MTMC drugs, substances that increase inflammation can synergistically augment chemical toxicity. Roth and coworkers (for review, see Ref. 69) demonstrated that a small dose of the inflammatory endotoxin lipopolysaccharide (LPS), which is without effect by itself, markedly enhances the hepatotoxic effects of aflatoxin B₁. A similar effect of LPS endotoxin occurs with other toxic chemicals and other target organs (for review, see Ref. 69). Stone et al.⁷⁰ have reported that LPS or the inflammatory cytokines TNF or IL-1 enhanced the cytotoxic effects of the SM stimulant 2-chloroethyl ethyl sulfide. Whether the results of coexposure to an inflammagen such as LPS or an intrinsic property of a toxic agent, the inflammatory response can augment sensitivity to chemical toxicities. Thus, inflammation associated with chemical toxicity is a target for anti-inflammatory MTMC.

MTMC Conclusions and Prospects

The MTMC hypothesis is supported by experimental evidence that distinct classes of drugs such as protease inhibitors and

TABLE III

ACTION OF ADENOSINE AGONISTS ON BIOLOGICAL RESPONSE

Situation	Response
Soman intoxication	Protection
NMDA _R -initiated inflammation	Inhibition
PARP	Inhibition
Asthma	Anti-inflammatory

PARP inhibitors have demonstrated efficacy against the nerve agent soman and the blister agent SM.^{33,36,41,53} Moreover, T-2 mycotoxin and SM injury can be alleviated by treatment with glucocorticoid hormones.^{60,61} By focusing on key biochemical signal pathways and cellular processes that ultimately contribute to pathologies associated with chemical toxicity, the MTMC hypothesis provides the possibility of developing single countermeasure drugs with prophylactic or therapeutic efficacy against distinct pathologies caused by multiple classes of toxic chemical agents.¹⁴ Furthermore, the MTMC concept may not be limited to chemical agents. Many biological toxins and infectious agents cause inflammatory responses that contribute to pathology. Finally, because MTMC addresses clinically recognized pathologies such as ARDS, collateral improvements in general health care and emergency medicine are possible.

Once one looks past the diverse chemistry, sites of action, and symptoms, common biochemical pathways may exist for many chemical threat agents. Even when threats extend to mixtures of agents and toxic industrial chemicals, or new threat agents evolve, such biochemical signaling pathways may remain more constant and amenable to medical intervention. Defining and interdicting these pathways provide strategies for developing civil defense and military preparedness against chemical threats. Of perhaps equal significance, the MTMC hypothesis provides an incentive for dialogue and cross-fertilization between diverse chemical defense, academic, clinical, and drug discovery research interests to combat chemical warfare and chemical terrorism. Using MTMC to blunt pathological actions of chemical agents is an attractive possibility.

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