REVIEW ARTICLE

The use of melatonin to combat mustard toxicity

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Submitted: 2008-05-20 Accepted: 2008-07-27

Key words: **bioterrorism; mustard toxicity; nitro-oxidative stress; melatonin; epigenetic**

Neuroendocrinol Lett 2008; **29**(5):614–619 **PMID:** 18987575 NEL290508R03

Abstract

Among the most readily available chemical warfare agents, sulfur mustard (SM) has been the most widely used chemical weapon. The toxicity of SM as an incapacitating agent is of much greater importance than its ability to cause lethality. Oxidative stress is the first and key event in the pathogenesis of SM toxicity. The involvement of inducible nitric oxide (iNOS) in SM toxicity, however, also leads to elevated nitrosative stress; thus, the damage caused by SM is nitro-oxidative stress because of peroxynitrite ($ONOO^-$) production. Once $ONOO^-$ is formed, it activates nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) leading to pro-inflammatory gene expression thereby promoting inflammation; additionally, $ONOO^-$ directly exerts harmful effects by damaging all biomolecules including lipids, proteins and DNA within cells. DNA damage is sensed by an important DNA repair enzyme, poly (ADP-ribose) polymerase (PARP); this enzyme repairs molecular damage by using nicotinamide adenine dinucleotide (NAD^+) as a substrate. Over-activation of PARP, due to severe DNA damage, consumes vast amounts of the respiratory coenzyme NAD^+ leading to a cellular energy crisis. This pathophysiologic mechanism eventually results in cellular dysfunction, apoptosis or necrosis. Therefore, classic antioxidants may have limited beneficial effects on SM toxicity. Melatonin is a multifunctional indolamine which counteracts virtually all pathophysiologic steps and displays significant beneficial effects against $ONOO^-$-induced cellular toxicity. Melatonin has the capability of scavenging both oxygen and nitrogen-based reactants including $ONOO^-$ and blocking transcriptional factors which induce pro-inflammatory cytokines. The delayed toxicity of SM, however, currently has no mechanistic explanation. We propose that epigenetic aberrations may be responsible for delayed detrimental effects of mustard poisoning. Therefore, as a putative epigenetic modulator, melatonin may also be beneficial to subjects with delayed toxicity of SM.

INTRODUCTION

Among the available chemical warfare agents, sulfur mustard (SM), also known as mustard gas, has been a widely used chemical weapon. Because of its devastating toxicity, its use during the World War I earned it the sobriquet "king of the battle gases". Other compounds such as nitrogen mustard (HN2) were developed during World War II, but found to be unsuitable as a munition. Soon after discovering HN2, it became the first non-hormonal agent used in cancer chemotherapy. A number of HN2 derivatives including cyclophosphamide (CP), ifosfamide (IF), mechlorethamine, melphalan and chlorambucil are valuable cytotoxic and radiomimetic agents for the treatment of cancer [22].

PROPOSED MECHANISM OF ACUTE TOXICITY

After several decades of research in our laboratory, it was revealed that SM, CP and other toxic agents share most of the same pathophysiologic mechanisms. Recent data consistently proves that reactive oxygen species (ROS) [42], nitric oxide ($NO^{\bullet}$) [26] produced by inducible nitric oxide synthase (iNOS) [41], and most importantly peroxynitrite ($ONOO^-$) [27,72] are involved in initial detrimental effects of all mustards [25,28].

$ONOO^-$ is *per se* not a radical but is a powerful nitrosating agent. $ONOO^-$ interacts with and covalently modifies all major types of biomolecules including membrane lipids, thiols, proteins and DNA [43]. $ONOO^-$ activates matrix metalloproteinases (MMPs) and triggers the expression of selectins and cellular adhesion molecules, via enhancing NF-κB activation, thereby promoting pro-inflammatory responses [60]. The mutagenic properties of $ONOO^-$-induced modified products have also been determined [20]. Single strand breakage can be induced by treatment with very low concentrations of $ONOO^-$ indicating that this agent is a potent inducer of DNA damage [73]. These observations suggest additional pathways by which $ONOO^-$ may be associated with not only elevated DNA damage but also impairment of DNA repair capacity [9]. $ONOO^-$ induces apoptosis and necrosis in cells. More highly elevated exposure of this agent is associated with necrosis rather than with apoptosis. In this mechanism, activation of the DNA repair enzyme poly (ADP ribose) polymerase-1 (PARP-1), a member of PARP enzyme family, mediates $ONOO^-$-induced necrosis [60,68]. PARP-1 detects and signals DNA strand breaks induced by a variety of genotoxic insults. Upon binding to DNA, strand breaks occur and, PARP transfers ADP-ribose units from the respiratory coenzyme nicotinamide adenine dinucleotide (NAD^+) to various nuclear proteins. From a physiological view point, PARP-1 activity and poly(ADP)-ribosylation reactions are implicated in DNA repair processes, the maintenance of genomic stability, the regulation of gene transcription, and DNA replication. An important function of PARP-1 is to allow DNA repair and cell recovery under conditions associated with a low level of DNA damage. In case of severe DNA injury, overactivation of PARP-1 depletes the cellular stores of NAD^+, an essential cofactor in the glycolytic pathway, the tricarboxylic acid cycle, and the mitochondrial electron transport chain. As a result, the loss of NAD^+ leads to a marked reduction in the cellular pools of ATP, resulting in cellular dysfunction and cell death via the necrotic pathway. This is known as "suicide hypothesis" of PARP activation and seems to be a regulatory mechanism to eliminate cells after irreversible DNA injury [59]. Experimental evidence has established that the PARP-1 pathway of cell death plays a pivotal role in tissue injury and organ dysfunction in mustard-induced acute toxicity [23,29].

BENEFICIAL EFFECTS OF MELATONIN AGAINST ACUTE SM TOXICITY

There is a large body of evidence that melatonin is major scavenger of both oxygen and nitrogen based radicals including $ONOO^-$ [1,21,45,47]. Melatonin has scavenging actions at both physiologic and pharmacologic doses. Not only melatonin but also several metabolites also have the capability to detoxify free radicals and their derivatives [46,63]. Melatonin also supports several intracellular enzymatic antioxidant enzymes [58]. Melatonin is significantly better than other antioxidants in this regard, e.g. it is more effective than vitamin E [5]. In many inflammatory processes, $ONOO^-$ rather than oxygen-based radicals is the predominant molecule which decides the fate of cells. Once formed, $ONOO^-$ cannot be scavenged by conventional antioxidants. As a multifunctional antioxidant, however, melatonin and its metabolites have unique features over the usual antioxidants including iNOS inhibition and $ONOO^-$ scavenging properties against mustard-induced acute toxicity [49,66,67,74].

Melatonin has been shown to ameliorate inflammation by blocking transcriptional factors and pro-inflammatory cytokines [39,50,69]. A large body of evidence confirms that these cytokines are capable of inducing formation of free radicals and promoting iNOS activity and transcriptional factor activation within cells. These events inevitably induce a vicious cycle of cellular damage. In the case of $ONOO^-$-induced DNA damage, PARP over-activates in an attempt to repair the genome, consumes NAD^+ as a substrate which causes an energy crisis within cells leading to their eventual necrosis. Preservation of NAD^+ and cellular energetics may be helpful for PARP to repair the DNA damage rather than blocking PARP. Melatonin preserves cellular energy production and ATP level in several pathologic circumstances [14,34,62].

PROPOSED MECHANISM OF DELAYED SM TOXICITY

Unfortunately, it is not clear how mustard gas causes severe multi-organ damage years after even a single exposure [3]. It is well known that most metabolites of mustard agents are excreted in the urine within a few weeks after exposure [56]. It is also well documented that mustard analogs such as CP and IF severely damage DNA and the gene environment. They have toxicity long after the initial exposure leading to cell death and an increased likelihood of cancer [55]. As noted above, the initial toxicity of mustards relates to a massive onslaught of highly reactive oxidizing and nitrosating molecules. For most mustard agents, once these changes occur the cellular effects essentially disappear. For SM, however, there are delayed progressive effects which render victims incapacitated for years [3,4,17,36,52]. The pathophysiologic mechanism of delayed SM toxicity currently has no mechanistic explanation [31].

Cells that are intoxicated with SM and are repaired by PARP-1 seem to be responsible of the delayed toxicity. These cells should be free of DNA damage, are able to divide but they also have either light to mild, but not severe damage. As explained previously, if the nuclear DNA in a cell is damaged, it is either repaired via several means including DNA repair enzymes [40] or the cell eventually dies [60,61]. However, if SM causes not only DNA damage but also alters epigenetic processes, this could explain, at least in part, the delayed effects of this warfare agent. We propose that epigenetic perturbations may be the underlying mechanism of the delayed effects of SM [7,35]. The term epigenetic describes the study of inheritable alterations in gene expression that occur in the absence of changes in genome sequence. This is in contrast to genetics, which deals with the transmission of information based on differences in DNA sequence. Therefore, epigenetic gene regulation requires molecular mechanisms that encode information in addition to the DNA base sequence and can be propagated through mitosis and meiosis. Our current understanding of epigenetic gene regulation involves three classes of molecular mechanisms: DNA methylation, histone modifications and DNA-binding proteins [6,71].

The chromatin structure is influenced by DNA methylation and DNA-histone interactions. The DNA-histone interaction is further influenced by covalent modification of histones and the action of DNA-binding proteins. The epigenotype can be transmitted from a parent cell to a daughter cell maintaining a specific epigenotype within cell lineages. Thus, the phenotype is a result of the genotype, the specific DNA sequence, and the epigenotype. The genotype must exist in a particular chromatin configuration, the epigenotype, which allows a secondary level of fine control over gene expression. The epigenotype shows far greater plasticity than the genotype, and it has been speculated that epigenetic errors could be a major contributor to human diseases [19]. Epigenotype is generally accepted as being less stable than the genetic system, and more sensitive to environmental [38], nutritional [16] and chemical toxicants [8,37].

Delayed toxicity of SM may occur in cells in which the genome has genetic and/or epigenetic mutations. If this is the case, delayed toxicity of SM may have a more diverse pathogenesis than acute toxicity and many beneficial approaches in treatment of acute toxicity may not function for delayed toxicity. Data based on the experience in Iranian veterans exposed to the agent during the Iran-Iraq conflict (1983–88) have clearly shown that delayed toxicity of SM is almost incurable even with extensive treatments [2]. The first report on the delayed toxic effects of SM poisoning in 236 Iranian veterans revealed that the delayed toxicity of SM persists in the respiratory tract (78%), central nervous system (45%), skin (41%) and eyes (36%). These effects were recorded between 2 and 28 months after exposure. In a study by Khateri *et al.* (2003) on 34,000 Iranians, 13–20 years after exposure to SM, the most common complications occurred in lungs (42.5%), eyes (39%), and skin (24.5%) [24]. Balali-Mood *et al.* (2005) described the toxic effects of SM poisoning in a group of 40 severely intoxicated Iranian veterans, 16–20 years after their initial exposure. The most commonly affected organs, in this study, were lungs (95%), skin (75%) and eyes (65%) [4].

POSSIBLE BENEFICIAL EFFECTS OF MELATONIN AGAINST DELAYED SM TOXICITY

Melatonin shows beneficial effects against SM-induced acute toxicity as a multifunctional antioxidant and $ONOO^-$ scavenging agent [57,67]. Also, several well-explained effects of melatonin seem to derive from epigenetic actions of the indolamine. For example, melatonin possesses genomic actions and regulates the expression of several genes. Melatonin influences cellular mRNA levels for antioxidant enzymes under both physiological conditions and during elevated oxidative stress [48]. The exact mechanism as to how melatonin stimulates antioxidant enzymes remains unclear. Consistent evidence however, suggests that melatonin modulates antioxidant enzyme activities via interaction with calmodulin, which in turn modulates epigenetic activation leading to gene expression [64,65]. A number of known anti-inflammatory effects of melatonin, such as selective inhibition of iNOS and/or cyclooxygenase-2 and MMPs clearly derive from melatonin and epigenetic cross-talk and modification through suppression of NF-κB binding [15] and/or p300-HAT expression within the nucleus [13].

Several oncostatic actions of melatonin are related to epigenetic regulation of gene expression. For example, melatonin inhibits telomerase activity and decreases mRNA levels of telomerase reverse (TR) transcriptase,

the catalytic subunit of telomerase via epigenetic mechanisms [32]. Expression of cyclin D1 protein, a crucial co-regulator of a variety of nuclear receptors, involves many pathophysiologic mechanisms including those involved in breast cancer. A key event for the anti-proliferative effects of anti-estrogens appears to be the down-regulation of cyclin D1 [10]. Melatonin has been shown to inhibit transcription of cyclin D1 expression [11] supporting the efficacy of melatonin on hormone-sensitive breast and prostate cancer [53]. Melatonin can also directly activate nuclear melatonin receptors and inhibit tumor growth in murine breast cancer cells [70]. The action of melatonin in advanced cancer patients [33] also seems to result from a combination of effects on histone modification and DNA methylation [12,30].

CONCLUDING REMARKS

Mustard gas is easy and inexpensive to produce, stable in storage and persistent in the environment. It inflicts casualties despite the use of respiratory or other types of protection and its potency is fairly high. Detection by odor is unreliable, although toxic levels can be noted; however, decontamination is difficult [54]. It causes delayed effects, producing no signs or symptoms until irreversible injury is inflicted leading to prolonged disability. Despite 75 years of research, there is still no antidote for mustard. This fact is especially crucial when we consider that probably at least a dozen countries have mustard in their arsenals today.

Melatonin has been administered in both physiological and pharmacological amounts to humans and animals, and there is widespread agreement that it is a non-toxic molecule [51]. In pregnant rats, maternal lowest-no-observed-effect-level (LOAEL) has been found to be 200 mg/kg/day and developmental no-observed-adverse-effect-level (NOAEL) is ≥ 200 mg/kg/day [18]. Melatonin is easily synthesized in pharmacologically pure form, non-patentable, inexpensive and affordable; therefore, it has a great potential to improve the public health [44] as a multi-tasking molecule. Melatonin has non-genomic, genomic and epigenetic actions; all these actions may be beneficial in both acute and delayed mustard toxicity.

Epigenetic therapy is a new and rapidly developing field in pharmacology. To date, most trials of epigenetic drugs have been conducted to evaluate their effects on cancer, many of which have shown promising results. Epigenetic drugs alone or in combination with conventional drugs may prove to be a significant advance over the conventional drugs used to treat both acute and delayed SM toxicity. Since epigenetic defects are thought to underlie a broad range of diseases, the scope of epigenetic therapy is likely to expand.

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