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Mini-review

Toxicity induced by chemical warfare agents: Insights on the protective role of melatonin

René Pita [a], José Marco-Contelles [b], Eva Ramos [c], Javier del Pino [c], Alejandro Romero [c,*]

[a] Chemical Defence Department, CBRN Defence School, Army Engineer School, 28240-Hoyo de Manzanares, Madrid, Spain
[b] Medicinal Chemistry Laboratory, Institute of General Organic Chemistry (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain
[c] Department of Toxicology & Pharmacology, Faculty of Veterinary Medicine, Complutense University of Madrid, 28040 Madrid, Spain

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ABSTRACT

Chemical Warfare Agents (CWAs) are substances that can be used to kill, injure or incapacitate an enemy in warfare, but also against civilian population in terrorist attacks. Many chemical agents are able to generate free radicals and derived reactants, excitotoxicity process, or inflammation, and as consequence they can cause neurological symptoms and damage in different organs. Nowadays, taking into account that total immediate decontamination after exposure is difficult to achieve and there are not completely effective antidotes and treatments against all CWAs, we advance and propose that medical countermeasures against CWAs poisoning would benefit from a broad-spectrum multipotent molecule.

Melatonin, a versatile and ubiquitous antioxidant molecule, originally discovered as a hormone synthesized mainly in the pineal gland, has low toxicity and high efficacy in reducing oxidative damage, anti-inflammatory effects by regulation of multiple cellular pathways and properties to prevent excitotoxicity, among others.

The purpose of this review is to show the multiple and diverse properties of melatonin, as a pleiotropic indole derivative, and its marked potential for improving human health against the most widely used chemical weapons.

Contents

1. Introduction

The North Atlantic Treaty Organization (NATO) defines chemical warfare agents (CWAs) as substances intended for use in military operations to kill, seriously injure or incapacitate people because of its pathophysiological effects [1]. The first CWAs were chemicals widely used in the chemical industry, such as chlorine and phosgene, two lung damaging agents. Afterwards, other CWAs, with no industrial use, were developed. This is the case of sulfur mustard, a blister agent used in World War I, or nerve agents, discovered just before the start and developed during World War II.

Sulfur mustard [bis(2-chloroethyl) sulphide] (Fig. 1) has been the most widely used CWA. As opposed to chlorine (Cl_2) and

* Corresponding author. Address: Department of Toxicology & Pharmacology, Faculty of Veterinary Medicine, Complutense University of Madrid, Avda. Puerta de Hierro s/n, 28040 Madrid, Spain. Tel.: +34 913943836; fax: +34 913943840.
E-mail address: manarome@ucm.es (A. Romero).

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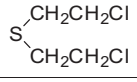
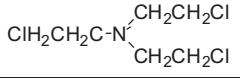
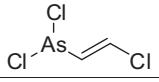
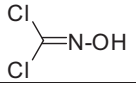
Sulfur Mustard (H, HS or HD)	Nitrogen Mustard-3* (HN3)	Lewisite (L)	Phosgene Oxime (CX)
			

*HN-3: Tris(2-chloroethyl)amine is the most important nitrogen mustard, produced for military purposes.

Fig. 1. Chemical structures of blister agents.

phosgene ($COCl_2$, carbonyl dichloride), which were gases at room temperature, sulfur mustard is a persistent agent that remains in the attacked area and affects not only the respiratory tract but the whole body surface. The blisters that appeared in affected troops in World War I are the reason why sulfur mustard and similar agents are called blister agents. Lewisites [dichloro(2-chlorovinyl)arsine] and nitrogen mustards (Fig. 1) were developed in the inter-war period and in World War II, respectively, however these blister agents did not provide any special advantages to sulfur mustard [2].

The 1994 and 1995 sarin terrorist attacks in Japan showed that CWAs should not only be considered in military, but also in terror attacks. The intentions and attempts to acquire CWA by jihadist terrorism make the chemical threat a main concern subject in terrorism studies and toxicological sciences.

Nowadays, total immediate decontamination after exposure is difficult to achieve and there are not completely effective antidotes and treatments against all CWAs, therefore more research is necessary in this field. This fact suggests us to study novel pharmacological approaches able to reduce CWAs-induce damage.

Melatonin (*N*-acetyl-5methoxytryptamine), a versatile and ubiquitous molecule, well-known as a potent indirect antioxidant and as a direct free radical scavenger [3–5], is also involved in vasomotor control and adrenal function, possesses antiexcitatory actions, regulates immune function and energy metabolism, including anti-inflammatory properties [6,7]. In mammals, three major melatoninergic membrane receptors have been identified. Among them, MT1 and MT2 are considered to represent the melatonin receptor system, and their physiological functions and pharmacological properties are well documented [8]. MT3 receptor has also been described and identified as a quinone reductase 2 [9]. However, nuclear binding sites/receptors also exist for this indole and have been identified as a further class of melatonin receptors [10]. The distribution of receptors and other binding sites indicates the remarkable pleiotropy of melatonin, which may potentially affect most of the cells, in addition to other receptor-independent mechanisms that may contribute to explain the different and tissue-specific functions of melatonin [11–13]. Melatonin is highly lipophilic, and consequently crosses easily cell membranes, including blood brain barrier (BBB) [14] allowing it to be administered either orally or intravenously, and to reach all subcellular compartments. The antioxidant capacity of melatonin is even more important owing to its ability to cross all morphophysiological barriers [15]. The subcellular distribution of melatonin allows it to interact with toxic molecules in the entire cell, reducing oxidative damage both in lipid and in aqueous cell environments. Moreover, its intermediate size is optimum for transportation across cellular membranes. However, rather little is known concerning the intracellular concentrations of melatonin.

Furthermore, in several comparative studies, melatonin has been shown greater protective effect than other antioxidants [16–19]. Taking into account melatonin's low toxicity and that patients treated with high doses of melatonin do not experience any harmful side effects [20], its potential spectrum for improving human health against CWAs seems to be wide.

Herein we summarize and hypothesize the possible protectiońs mechanism of melatonin against several CWAs, classified in several groups such as nerve agents (I), vesicants or blister agents (II), lung damaging agents (III), and cyanides (''blood agents'') (IV).

2. Toxicology of nerve agents and beneficial effects of melatonin

Nerve agents are organophosphate (OPs) compounds that were initially synthesized by German scientists prior to World War II and are one of the deadliest compounds known to man [21]. Furthermore, these agents present higher mammalian toxicity than their OP biocide homologues. The four major OP nerve agents are tabun, sarin, soman and VX (Fig. 2). They differ from one another in their potency and volatility, as well as in their ability to cross the BBB and to exert central nervous system (CNS) toxicity. Soman is the most potent of the four [22]. Nerve agents have both chemical names as well as 2-letter NATO codes. There are two main series: ''G'' agents (''G'' stands for ''German'') and ''V'' agents (''V'' stands for ''venomous''). ''G'' agents include tabun (*O*-ethyl *N*,*N*-dimethyl phosphoramidocyanidate, GA), sarin (*O*-isopropyl methylphosphonofluoridate, GB) and soman (*O*-pinacolyl methylphosphonofluoridate, GD), among others (see Fig. 2). The most important ''V'' nerve agent is *O*-ethyl *S*-2-diisopropylaminoethyl methyl phosphonothiolate, known as VX. ''G'' and ''V'' agents are both liquids at room temperature, but ''G'' agents are more volatile than ''V'' agents, thus ''G'' agents are non-persistent CWAs, while ''V'' agents are considered persistent agents [23].

The different OPs are absorbed nearly through all body surfaces, but mainly through the skin, eyes, and respiratory tract [24–26]. Due to their high toxicity, once absorbed, they produce systemic effects more rapidly than their biocide homologues. Their main toxicological mechanism of action is the powerful irreversible inhibition of peripheral and brain cholinesterases, which produce a cholinergic syndrome that may lead to death by asphyxia caused by airway obstruction, respiratory muscles paralysis, and respiratory depression at CNS level [27,28]. If untreated, the cholinergic overstimulation triggers peripheral or central muscarinic and nicotinic signs. In addition, increased acetylcholine levels initiate seizures *via* the overactivation of muscarinic receptors [28–30]

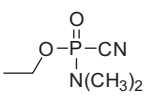
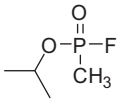
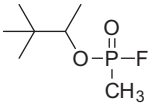
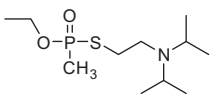
Tabun (GA)	Sarin (GB)	Soman (GD)	VX
			

Fig. 2. Chemical structures of nerve agents.