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Chemical and Pharmacological Mechanisms of Plant-Derived Neurotoxins

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6.1 Introduction

The nervous system (NS) comprises the central and peripheral divisions. While the central nervous system (CNS) consists mainly of the brain and spinal cord, the peripheral nervous system (PNS) is subdivided into the somatic nervous system and autonomic nervous system (ANS). The ANS is further subdivided into the sympathetic and parasympathetic systems. In every component of the NS, information passes from one cell to another or from the NS to the muscular system by means of neurotransmitter molecules that are secreted by neurons. All neurotransmitters have a general mode of action, involving binding to a receptor and altering the target cell's response to a particular signal (the stimulus). The molecular interaction between a neurotransmitter and its receptor induces a conformational change, which, in turn, changes the electrochemical properties of the nerve cell, promoting entry and/or exit of some electrolytes and generation of an action potential, which enables communication between cells. A number of neurotransmitters are known and well understood in terms of their molecular and biological activities [1]. It is also known that exogenous molecules can enter the body, traverse all the way to the NS, and bind to either receptors or their counter-ligands. The binding of exogenous molecules by either means could promote undesirable effects, some of which exert toxic metabolic responses. In a unique mechanism, nerve agents bind to the enzyme acetylcholinesterase (AChE) and inhibit its ability to recycle the neurotransmitter acetylcholine (ACh) across nerve junctions. There are two types of ACh receptor (AChR), namely nicotinic (N) and muscarinic (M) receptors. Among other organisms, plants are known to be sources of an enormous number of neurotoxic molecules [1]. We highlight the most potent neurotoxins related to well-established modes of neurotransmission.

6.2 Nerve Agents

Under normal nerve impulse transmission, in the CNS, a presynaptic neuron secretes the neurotransmitter ACh into the synaptic cleft, enabling the neurotransmitter to interact with its cholinergic receptors on the postsynaptic membrane surface. This interaction leads to opening of the sodium channels on the postsynaptic neuron, followed by generation of an action potential and propagation of the impulse along the axon of the postsynaptic neuron. In so doing, the message spreads from one nerve cell to the next. In the PNS, when the action potential reaches a junction between a motor neuron and a muscle (a neuromuscular junction), a similar mechanism occurs via the same neurotransmitter across the neuromuscular junction and the impulse results in a muscular response related to movement depending on the nature and goal of the stimulus. After the

action potential passes, the enzyme AChE cleaves ACh into acetyl and choline, thereby stopping its interaction with its receptor in order to block the sodium channel and the subsequent effects of the action potential. When the enzyme is bound to nerve agents, its ability to break the neurotransmitter is blocked, promoting continued ACh–AChR interaction and a progressive action potential, leading to undesirable nerve and muscular effects.

The history of nerve agents dates back to the early 1930s with the discovery of tabun by Gerhard Schrader, who was working to discover novel insecticides. Other prominent nerve agents discovered during these earliest stages include sarin, cyclosarin, and soman, all of which are organophosphates (OPs) and were deployed as biological weapons during World War II [2]. Another well-known OP is diisopropyl fluorophosphate, which is used in the treatment of glaucoma and as an inhibitor of AChE in the parasympathetic division of the PNS.

The physical and chemical properties of OP nerve agents are similar. As a general property, all OP nerve agents are volatile liquids with varying faint odors ranging from fruit juice to camphor [3].

6.3 Chemical Mechanisms of Neurotoxicity Induced by Organophosphate Nerve Agents

By binding to the enzyme AChE, poisonous agents such as OP compounds prevent its ability to hydrolyze its substrate, ACh, at the synapse. ACh then remains in higher than required concentrations and promotes progressive nerve action potentials that result in nerve hyperactivity and fatigue [4]. Sarin (propan-2-yl methylphosphonofluoridate) has been used as chemical warfare because of its irreversible binding activity to AChE, which causes permanent enzyme inactivation. Sarin inhibition starts by phosphorylation of the hydroxyl group of the serine residue on the active site of the enzyme, increasing its half-life from hours to days. In addition, as a result of increased phosphorylation, the enzyme undergoes a process known as aging, which is characterized by the loss of alkyl groups with subsequent resistance to ACh-mediated cleavage [2, 5]. The clinical manifestations of sarin-induced neurotoxicity vary depending on the type of cholinergic receptor involved, but they generally include pinpoint pupils; blurred and dimmed vision; salivary, sweat, bronchial, and lacrimal gland hypersecretions; cardiovascular upset; seizures; ataxia; musculoskeletal distortion; and even paralysis [6]. These signs are indicative of acute and chronic neurotoxicity and are caused by overaccumulation of ACh in the synaptic junctions, leading to hyperstimulation of the individual's NS [5, 7]. Exposure to sarin can cause death within a few minutes or hours, making it historically one of the most potent chemical warfare agents.

On the other hand, soman (*O*-pinacolyl methylphosphonofluoridate) is a synthetic nerve agent whose mechanism of action involves binding AChE as well

as butyl cholinesterase, which is a plasma cholinesterase [2]. On binding to AChE, soman phosphorylates the serine residues and loses is phosphoryl group, forming methylphosphonic acid. The latter can be decomposed via hydrolytic activity or the action of oximes, regenerating the enzyme.

Unlike sarin and soman, which contain a fluorine substituent, tabun contains a cyanide substituent. Other names for tabun include *N*′-*N*-phosphoramidophosphate or ethyl *N*′-*N*-dimethylaminocyanophosphate, among others. The acute toxicity induced by tabun is characterized by seizures, salivation, miosis, muscle fasciculation, and paralysis, all of which result from overstimulation of both the muscarinic and nicotinic AChRs in the entire body [8]. Exposure to lethal doses of tabun through the skin, eyes, and gastrointestinal tract can cause death in less than 10 minutes [3]. After the nerve agent accumulates in the NS it can undergo spontaneous or enzymatic hydrolysis via cleavage of the P–CN bond [5].

6.4 Mustards

Mustards are vesicant agents that not only cause blistering of the skin and mucous membranes but also damage the eyes and respiratory tract at high doses [9]. Sulfur mustard (2,2′-dichloroethyl sulfide, HD) and nitrogen mustard (*N*-methyl-2,2′-dichlorodiethylamine) both exert toxic effects, but the former is more potent than the latter [10]. Mustards are colorless to light yellow viscous liquids that smell like garlic or fish in high concentrations [11–14]. Figure 6.1 shows the absorption and distribution pattern of sulfur mustard on contact with the skin.

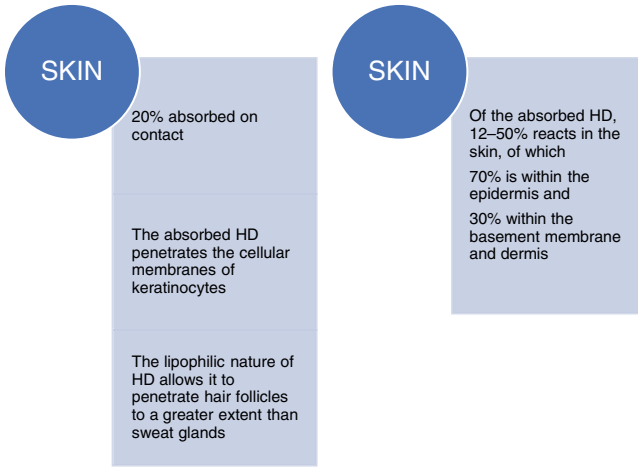

Figure 6.1 Penetration of sulfur mustard (HD) into skin through hair follicles. (*See color plates for the color version of this figure.*)

The greasy nature of HD makes it more attractive to the lipophilic parts of the body, such as hair follicles [15–17]. After exposure to HD, DNA adducts, as measured by monoclonal antibodies, were easily seen within keratinocytes, yet no adducts were seen within the dermis [16]. Systemic absorption of HD is rare because it is hydrolyzed within organs such as the eyes, skin, and lungs [16]. The vaporized form of HD causes more major injuries than the liquid form. Human contact with the liquid form of HD is rare since most chemical warfare agents are usually packaged in and used with explosives.

6.4.1 Effect of HD on Skin

Figure 6.2 shows how HD affects the skin.

6.4.2 Effect of HD on Other Organs

According to the literature, HD has a profound adverse effect on various organs of the body, including the eyes, skin, and soft organs, such as the lungs, liver, spleen, and kidneys [15–17]. It is important to note that the human body is weakened when the liver is compromised [18]. Both physical weakness and biological weakness may be experienced in the course of HD acting on soft organs [19]. Figure 6.3 summarizes the effects of exposure to HD on various organs.

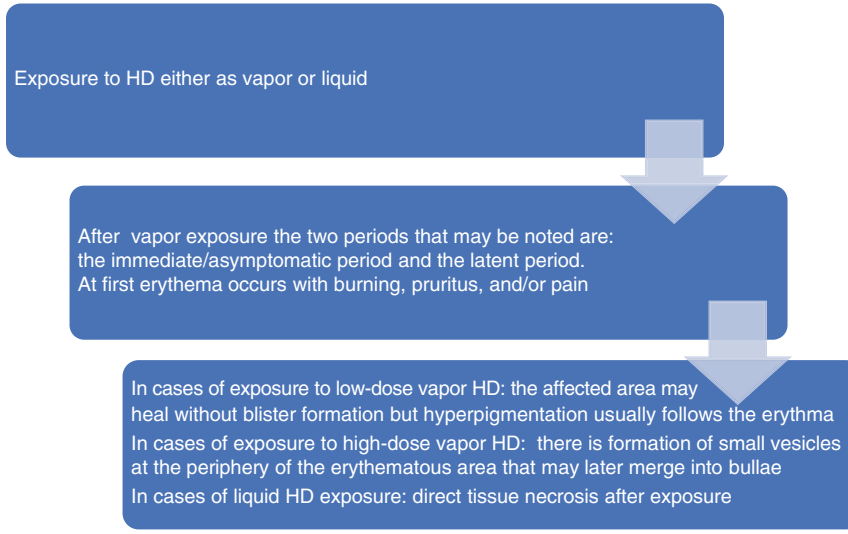

Figure 6.2 Skin exposure to sulfur mustard (HD). (*See color plates for the color version of this figure.*)

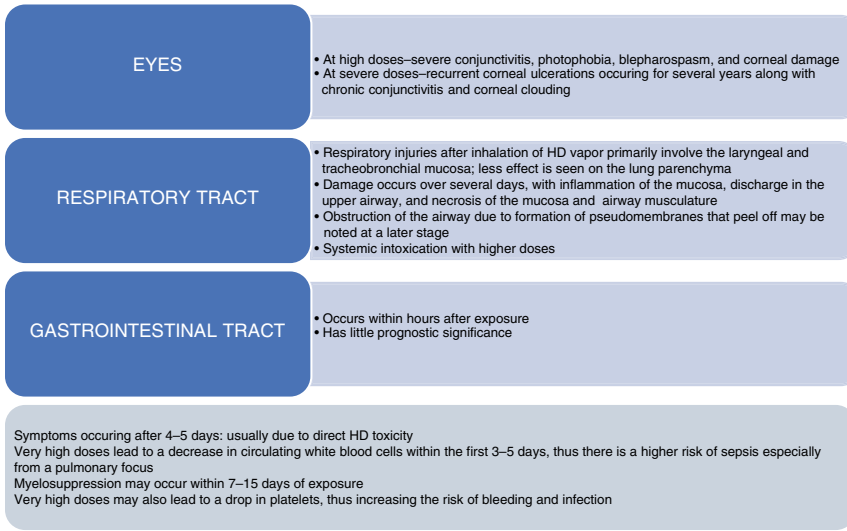

Figure 6.3 Adverse effects of sulfur mustard (HD) on various organs. (*See color plates for the color version of this figure*.)

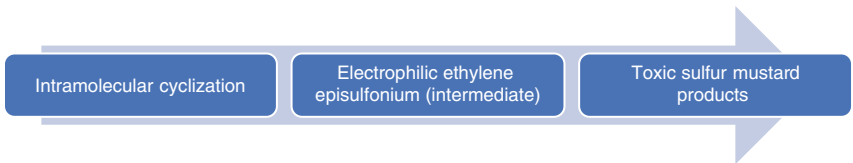

Figure 6.4 The activation process of sulfur mustard (HD). (*See color plates for the color version of this figure*.)

6.4.3 The Activation of HD

Despite being lipophilic, HD requires reaction with an aqueous medium for biotransformation [14] (Figure 6.4).

The avidity of compounds for the electrophilic products produced by hydrolysis of HD is determined by the availability of electrons within the molecules as well as the functional groups in the molecules that increase electron availability and decrease reactivity. There is similarity between the rate of alkylation and the rate of hydrolysis by HD, but the products of alkylation exhibit greater stability than those of hydrolysis [14, 15, 17, 20, 21].

6.4.4 Mechanism of Action

There are several mechanisms proposed in the literature for the action of HD.

It is known that thiols are one of the body's main defense mechanisms against electrophilic stress (ES) and reactive oxygen species (ROS). Researchers have also proposed that a significant proportion of HD toxicity is secondary to ES or ROS with depletion of cellular detoxifying thiols, including glutathione. Note that microfilamentous proteins, which maintain the cytoskeletal and structural integrity of the cell, can induce apoptosis or necrosis through activation of endonucleases, proteases, and/or phospholipases, and thus can induce DNA and/or membrane damage.

HD can cross-link DNA and produce single-stranded DNA breaks since they are both bifunctional alkylating agents [16, 17, 22, 23].

The molecular mechanism involved in HD-induced epidermal cell injury and death is not completely understood yet. Following DNA alkylation, with induction of DNA repair of strand breaks/apoptosis, the enzyme poly(adenosine diphosphate-ribose) polymerase (PADPRP) is activated [15, 17]. In enzymatic reactions with a number of nuclear proteins, PADPRP utilizes nicotinamide adenine dinucleotide (NAD+) as a substrate in these reactions, as shown in Figures 6.5 and 6.6.

Increased levels of proteases are proposed to play a role in HD-induced blister formation and cellular damage.

In the absence of steric effects, most of the neutrophilic sites are susceptible to HD alkylation [16]. Runs of guanines are present in only 1.3% of the human genome and act as preferential sites for HD alkylation; on the other hand, a significant number of oncogenes and viral oncogenes are rich in guanine, thus explaining why some tumors – particularly virus-induced tumors – respond to this class of drugs [23, 24].

Since there is a preference for a specific site in the DNA binding, this also means that some structural proteins, adhesion molecules, cytokines, and/or enzymes are affected whereas others are not. The interstrand cross-links are major inhibitors of DNA synthesis, with comparatively minor effects on total protein and RNA synthesis, which are the primary targets in rapidly proliferating cells. There is a variation in the sensitivity of different cells to such agents. For example, cells in

Figure 6.5 A simple process illustrating nicotinamide adenine dinucleotide (NAD+) depletion. (*See color plates for the color version of this figure.*)

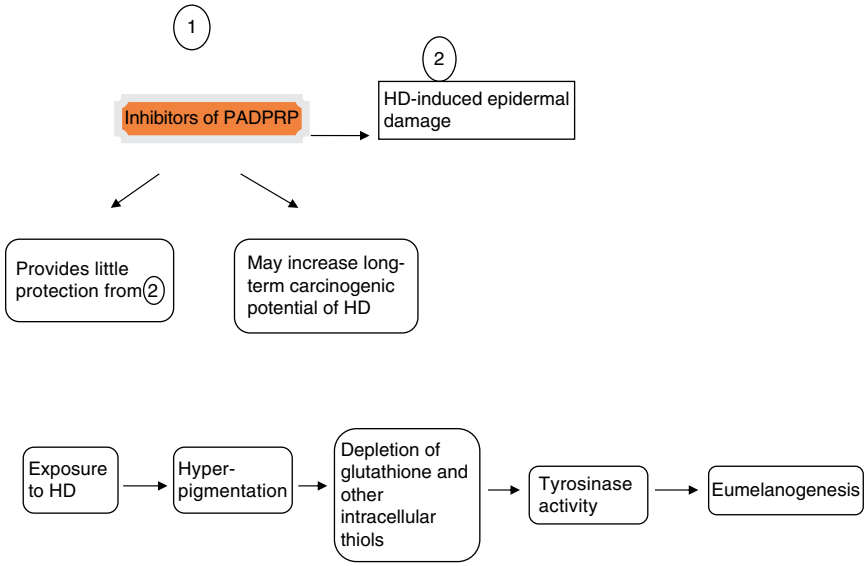

Figure 6.6 Sulfur mustard (HD) blister formation as a result of increased levels of protease. PADPRP, poly(adenosine diphosphate-ribose) polymerase. (*See color plates for the color version of this figure.*)

the basal cell layer of the epidermis that are transiently amplifying are expected to be more sensitive to these agents; this is also the case for stem cells, which need to maintain a low cytoplasmic/nuclear ratio. The sensitivity of stem cells to these agents can be explained by unbalanced growth along with an increase in the cytoplasmic/nuclear ratio as a result of marked inhibition of nuclear DNA synthesis, cytoplasmic proteins, and RNA [24]. In addition, melanogenesis may be enhanced by DNA repair enzymes that are upgraded by enzymes that damage DNA [16].

6.5 Plant Natural Neurotoxins

A variety of plants used for pharmaceutical, nutritional, and other industrial purposes contain a wide range of toxic compounds from various plant parts at different levels. A range of groups of compounds from various plants have been implicated in a number of neurotoxic pathologies.

Non-proteinogenic amino acids and glycosides are examples of groups of compounds that are notorious for their neurotoxicity [1].

Plants synthesize a significant range of non-proteinogenic amino acids for ecological purposes. Although a good number of these secondary amino acids are

utilized by some plants for physiological development, a wide range are deployed as weapons in the fight against ecological competitors. The majority of these amino acids resemble the endogenous amino acids of the human body and therefore are comparatively active toward their de novo receptors in vivo.

The genera *Lathyrus* and *Panax* constitute some of the plant groups known to possess neurotoxic amino acids [25–27]. An unusual non-protein amino acid, β-*N*-oxalyl-L-α,β-diaminopropionic acid (β-ODAP), is a strong neurotoxic molecule produced by these plants and has been found to mediate its activity via molecular interaction and agonistic effects with non-*N*-methyl-D-aspartate (NMD) receptors. As it binds to these receptors, it induces excitotoxicity by inhibiting exchange of L-cystine and L-glutamate via the system $x_c(-)$ transporter, an amino acid transporter of the glycoprotein-associated amino acid transporter (gpAT) family [26, 28]. Accumulating amounts of β-ODAP in the CNS cause the slowly spreading disease neurolathyrism; in addition to its structural similarity to L-glutamate, the compatibility of β-ODAP at multiple sites prompts its multitarget-mediated neuroaccumulation and toxicity [29].

L-Glutamate is a major excitatory neurotransmitter and precursor of another neurotransmitter, γ-aminobutyric acid (GABA), the principal inhibitory neurotransmitter. Glutamate receptors are both synaptic and non-synaptic, residing in the membranes of neurons as well as in neuroglia [30]. Glutamate interaction with its receptors mediates postsynaptic neuronal excitation and plays a major role in neural communication, memory formation, and regulatory processes. Chronic overactivation of glutamate receptors is implicated in a number of neuropathological conditions as a result of excitotoxicity. The accumulation of glutamate or its analogs such as β-ODAP induces prolonged activation of *N*-methyl-D-aspartate receptor (NMDARs), leading to high levels of calcium ions (Ca^{2+}) following their massive influx into the postsynaptic cell. The accumulation of calcium ions induces a number of biochemical cascades that promote neuronal damage and subsequent death [31]. Excessive intracellular Ca^{2+} promotes neuronal death by activating several forms of hydrolytic enzymes, including proteases (such as calpains), caspases, nucleases, and lipases [32]. In addition, high levels of calcium promote activation of nitric oxide synthase (NOS) and generation of free radicals, with consequent overwhelming oxidative stress leading to neuronal death [32]. The clinical syndromes associated with β-ODAP accumulation and its excitotoxic effects include convulsions, trauma, ischemia, lower limb paralysis, gluteal muscle emaciation, and long-lasting seizures [29].

In the early 1960s, *Lathyrus cicero* and *Lathyrus sativus* were found to produce the amino acid homoarginine, also known as L-2-amino-6-guanidinohexanoic acid [33]. The neurotoxic effect of this compound is linked to its propensity to increase ammonia levels while inhibiting uptake of ornithine and lysine in the brain [34].

Studies have shown that homoarginine binds neural nitric oxide synthase (nNOS), the enzyme that catalyzes the synthesis of the signaling molecule nitric oxide (NO), normally from L-arginine [31]. NO is a signaling molecule in the CNS and PNS that acts by increasing the activity of soluble guanylate cyclase, which in turn synthesizes cyclic guanosine monophosphate (cGMP) [35, 36]. Homoarginine activates the G-protein-coupled receptor GPRC6A in a similar manner to L-arginine. In the nervous tissue, this activation is associated with increased levels of calcium ions [36], which may account for Ca^{2+}-induced neurotoxicity and mortality.

Caramboxin (CBX) is another NMDAR agonist, with a similar mechanism of action to that of β-ODAP. The compound is produced by plants of the genus *Averrhoa* (commonly known as starfruit, *Averrhoa carambola*). The earliest neurotoxic effect was described in Malaysia following intraperitoneal administration of crude extracts; preliminary findings showed convulsions, unconsciousness, and immediate death, suggesting that the starfruit plant produces a depressant metabolite [37]. CBX is known to act on the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) ionotropic receptor, mediating convulsant, excitatory, and neurodegenerative effects [38].

Structurally, CBX resembles the amino acid phenylalanine, with slight modifications on the aromatic ring, which contains hydroxyl, carboxyl, and methoxy groups. CBX is both a neurotoxin and a nephrotoxin owing to its ability to interact with oxalic acid in the fruit, suggesting that consuming star fruits poses neurotoxic and nephrotoxic risks not only to renally impaired individuals but also to normal individuals [39].

Very well described neurotoxic amino acids from the genus *Polygonatum* include L-α,γ-diaminobutyric acid, L-γ-aminobutyrine, and L-2,4-diaminobutanoic acid (DAB) [34]. Exposure to this amino acid is associated with mass accumulations in the liver, followed by chronic ammonia toxicity to the CNS [40]. The mechanism of action of DAB occurs by competitive inhibition of ornithine carbamoyltransferase, an enzyme catalyzing the formation of citrulline from ornithine and carbamoylphosphate in the urea cycle. This reaction is essential in the detoxification of ammonia, thus inhibition by DAB promotes the increase of ammonia in the blood and the brain, resulting in neurolathyrogenic symptoms such as tremors, hyperirritability, and convulsions, as consequences of liver and brain damage [40].

6.6 Plant Glycosides

Glycosides are a group of abundant natural products in plants as well as in microorganisms. Fabaceae, Rosaceae, Leguminosae, Linaceae, and Compositae are among the well-known families producing cyanogenic glycosides. Cyanogenic

glycosides have been used as a chemotaxonomic group of compounds and they are found extensively in many edible plants such as peaches, cherries, bamboo, cassava, coco yam, beans, and cashew. As toxins, glycosides are implicated in a wide range of pathological conditions, including goiter spastic paraparesis and tropical and ataxic neuropathy [41]. The potential risk of cyanogenic glycosides is posed by their ability to produce hydrocyanic acid or hydrogen cyanide (HCN) [42]. Well-known examples of cyanogenic glycosides include lotaustralin, amygdalin, linamarin, taxiphyllin, and dhurrin, among many others; these are produced in variable amounts among plant species [41].

Cyanogenic glycosides are biosynthesized as defensive secondary metabolites from one of the five proteinogenic amino acids – L-valine, L-isoleucine, L-leucine, L-phenylalanine, and L-tyrosine – as well as from one non-proteinogenic amino acid, cyclopentenyl-glycine [42].

One of the common neurological diseases caused by cyanogenic intoxication is an upper motor neuron disease known as *konzo*, which is most widely known to be due to consumption of cassava [43]. The disease afflicts mostly children and women of child-bearing age and is marked by irreversible non-progressive symmetric spastic paraparesis [43]. The cyanide produced by cyanogenic glycoside mediates toxicity by halting cellular oxidative respiration via cytochrome oxidase a_3 inhibition of the terminal enzyme in the respiratory chain [42].

6.7 Conclusion

Plants are well known to contain medicinally important phytocompounds, but little is known about their synthesis of metabolites that lead to neurotoxins and other agents affecting the NS. It is important to note that the plant kingdom is capable of enhancing adverse effects on the NS; at the same time, it can produce antidotes. If no direct antidotes can be produced, synthetic ones from the plant kingdom can also be made.

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