Acute Carbamate Insecticide Intoxication in Forensic Medicine

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Carbamates are potent cholinesterase inhibitors derived from carbamic acid but compared to organophosphates their action is reversible thus making them less dangerous. Carbamates are frequently used as insecticides the most known in Romania being carbofuran (Furadan) secondary to a famous singer's suicide using this substance. In the current paper the authors analyse the severity, mechanism of action and therapeutic methods used in carbamate insecticide intoxication based on one case of accidental poisoning secondary to contaminated beer ingestion. To our opinion, carbamates exhibit a different toxicity than the organophosphates, with a more brutal, life-threatening behaviour than previously considered.

Keywords: carbamates, insecticides, cholinesterase inhibitors, poisoning

Carbamates are being used in medical therapeutics since ancient times and at the end of the nineteenth century, Physostigmine (eserine) extracted from *Physostigma venenosum* was recommended in the treatment of glaucoma. Most of what is known about the action of carbamate insecticides is due to the study of Physostigmine and its synthetic analogs. Its property of inducing myosis and other symptoms was then attributed to the urethane nucleus. This observation led to the synthesis of numerous molecules based on urethane nucleus, the most known being Neostigmine, initially used for transit acceleration and later for myasthenia treatment [1, 2].

In medical therapeutics, carbamates are nowadays used for treating myasthenia gravis, glaucoma, anticholinergic poisoning, supraventricular arrhythmias, and as tranquilizers.

Carbamates are largely used as pesticides (herbicides, fungicides and insecticides). Esterified (heterocyclic) carbamates used as insecticides have the common formula ROC(O)N(CH3)R' where R can be an alcohol, an oxime, or a phenol and R' hydrogen or a methyl group. Carbamate insecticides act by reversibly inhibiting cholinesterases in both insects and mammals [3].

In Romania, carbofuran was used as an insecticide (Furadan) on a large until it was forbidden due to its high toxicity to humans but other carbamate insecticides are still commercialized, for example metiocarb (Mesurol 2 RB[®]), bendiocarb (Ficam WP80[®]) and methomyl (LANNATE 20 SL[®]). All 3 examples are approved for use and catalogued as class H301 toxics (toxic if swallowed). Among side effects in case of swallowing they can produce bradycardia, sweating, convulsions, nausea, lachrymation, salivation, vomiting, diarrhoea, miosis, hypotension, bronchial hypersecretion, myoclonus, respiratory paralysis, somnolence, coma, respiratory failure, hypothermia, fibrillation, and spasm.

A male patient, aged 36 years, was admitted in Intensive Care Unit (ICU) with Glasgow score 6 coma. Patient's relatives called emergency rescue service (SMURD) after finding him in another room having difficulties breathing and with an altered state of consciousness. Relatives declared an acute onset of symptoms after consuming caned beer that he bought from a local shop approximately

120 minutes before. Ingested quantity was estimated at 900 mL (100 mL left in one can). The patient was already comatose when SMURD arrived (14 minutes after the call was placed) and presented myosis, bradycardia (38 beats/ minute), hypotension, respiratory distress (bilateral crepitations), and fasciculations. Glasgow coma score was calculated at 6 and the patient was intubated and mechanically ventilated. Blood analysis upon hospital admission revealed normokalemia, normocalcemia, normal phosphate levels, reduced alkaline reserve (11 mmol/L), leukocytosis (14650/ µL), hyperglicemia (147 mg/dL), haemoconcentration, rhabdomyolysis, increased hepatic enzymes (aspartate transaminase 139 U/L, alanine aminotransferase 127 U/L). Bilateral perihilar haziness was observed on the chest X-ray, thus confirming respiratory distress. Drug over dosage was suspected but testing for common drugs proved negative. Serum cholinesterase level was decreased to 1174 U/L (normal value Synevo laboratories 5320-12920 U/L). Organophosphate poisoning was suspected based on clinical symptoms and decreased serum cholinesterase level. Gastric content (gastric lavage) was submitted for analysis at the Institute of Forensic Medicine from Iasi, Romania and toxicological analysis revealed the presence of methomyl, a carbamate insecticide. County Police initiated an investigation for manslaughter and took samples from patient's house. Among samples, two cans of beer (one empty, the other one with 100 mL left) were submitted for analysis at the National Institute of Forensic Medicine from Bucharest, Romania, the results being similar to the gastric content as methomyl was present in the fluid left in the second beer can. Atropine treatment was initiated 2 hours and 25 minutes after arrival (a total of 68.7 mg, initial bolus followed by syringe pump). The patient was atropinised for 32 hours and needed mechanical ventilation together with sedation for 3 days. Atropinisation was considered efficient and sufficient when the patient developed reactive mydriasis associated to dryness of mucosa. Total hospitalization time was of 12 days. The patient was discharged with residual neurologic disorders (muscle weakness).

Discussions

Carbamates absorption is significantly influenced by the excipient and the route of exposure, the importance of

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these factors varying among the totality of these products. Carbamates are readily absorbed as they pass through the gastrointestinal tract. It should be noted that as the carbamates pass very little through the blood-brain barrier, the effects on the central nervous system (CNS) are be less severe than those observed with organophosphates. Metabolization and elimination are fast, with no evidence of bioaccumulation of carbamates [4].

Generally, all tissue can metabolize xenobiotics to some degree, but the liver is the main organ. Most enzymes are located in the liposoluble fraction of the endoplasmic reticulum membrane but mitochondria, lysosomes, cytosol may also contain such enzymes. Carbamates biotransformations were divided into two distinct phases. Phase 1 reactions usually transform xenobiotics into a more polar metabolite by the addition or unmasking of a functional group, which results in functionalization of the molecule. If phase I derivatives are sufficiently polar, they can already be excreted. However, in most cases, phase I products undergo further biosynthetic reactions, known as phase II reactions where endogenous substrates such as glucuronic acid or an aminoacid combine with them to form a highly polar conjugate. A common feature of phase II reactions is the need for an activated intermediate (cofactor or drug). In general, with respect to the carbamates, phase I reactions are of the oxidation or hydrolysis type, and phase II reactions are of glucuronidation or sulphoconjugation type, resulting in the formation of a hydrosoluble product, excretable via the kidneys [5, 6].

Toxic action modes

Carbamates are direct and rapidly-reversible acetylcholinesterase (AChE) inhibitors by carbamylation of the esterase (active) site of these enzymes. They are fixed on a site analogous to that of acetylcholine, borne by a serine, and are hydrolysed similar to the natural substrate.

According to Corbett et al. [6] the enzymatic process can be represented as follows:

$$\stackrel{ki}{\vdash} EOH + AB \stackrel{k1}{\leftarrow} EOH.AB \stackrel{k2}{\rightarrow} EOA \stackrel{k3}{\rightarrow} EOH$$

$$\stackrel{+}{H} + B \stackrel{+}{A^{k-1}} + OH$$

where: EOH – acetylcholine; AB – carbamate; EOA carbamylated enzyme; B - alcohol, an oxime or a phenol; A - the denatured heterocyclic carbamate.

The first part of the equation, reversible formation of EOH-AB complex takes place almost instantaneously. This EOH-AB affinity can be characterized by a dissociation constant Ka = (K - 1)/(K + 1), the smaller Ka is, the greater the affinity.

The second part of the equation (EOA formation) characterizes the carbamylation of a specific site. It is an ester site carried by a serine and able to be phosphorylated or acetylated (covalent bond).

The last part of the equation is a hydrolysis reaction where decarbamylation of acetylcholinesterase is observed. This hydrolysis is 105 to 106 times faster for a methylated compound than for a dimethyl compound. However, this large difference will be reflected only by a 1-3 factors on their Ki (reactivated with the enzyme). Decarbamylation restores AchE molecule to its initial state.

Carbamylation is reversible for the enzyme, but not for the carbamate, which loses its activity in this process by cleavage and hydrolysis. Phosphorylation of the same site of this enzyme can be irreversible depending on the degree of lipophilicity of the organophosphate (high in alkyl-thiophosphates) [7-9].

This reversibility, as opposed to organophosphates, is responsible for a less dangerous poisoning.

The toxicological and insecticide action of carbamates and organophosphates is related to the inhibition of true AchE (acetylcholine acetylhydrolase) of the central nervous system, muscles, red blood cells than on pseudocholinesterase (butyrylcholinesterase, acylcholineacylhydrolase) inhibition in the central nervous system and plasma [7, 10].

These cholinesterases serve to quickly hydrolyse acetylcholine fixed to the receptor into choline and acetic acid, in order to stop the stimulation and restore receiver sensitivity. Thus, the accumulation of non-hydrolysed acetylcholine at post-ganglionic muscarinic receptors, nicotinic receptors in the neuromuscular synapse, ganglia of the autonomic nervous system, and central cholinergic synapses explain the clinical symptomatology during intoxication with carbamates [6, 7, 10].

Toxicodynamics

Acute toxicity is related to its anticholinesterastic effects. Cholinergic receptors can be classified into two categories, based on their susceptibility to different drugs, muscarine, nicotine, atropine and curares, but four entities are useful for understanding carbamate intoxication.

Post-ganglionic synapses of the parasympathetic system

Stimulation of the parasympathetic neuroeffector synapse is at the origin of the so-called muscarinic effects explaining respiratory, cardiac, digestive, urinary, and ocular symptoms. Dyspnea, bronchorrhea, pulmonary edema, with, according to their degree of intensity, signs of respiratory distress, are noted both in conscious an unconscious patient who also present hypersialorrhea, nausea, vomiting, diarrhoea, urinary and faecal incontinence [10]. The pupils are in tight miosis, and bradycardia and hypotension occur in varying proportions.

The skeletal neuromuscular synapses

Symptoms related to the neuromuscular junction are classically referred to as nicotinic in relation to the "dose" of acetylcholine (small quantity - receptor stimulation, high doses - receptor blockage) [11]. According to the case one can note fasciculations, contracture, muscular weakness or paralysis. These anomalies also affect the diaphragm and contribute to possible respiratory distress.

Preganglionic synapses of the sympathetic and parasympathetic nervous system

These synapses are stimulated by nicotine, but are insensitive to muscarine, atropine or curares, except at high concentration. In case of carbamate poisoning, stimulation of these receptors will induce effects classically associated with nicotinic effects (i.e., tachycardia, mydriasis, paleness, hyperglycaemia) [10, 11].

Cholinergic synapses of the central nervous system

Disturbances in the functioning of cholinergic synapses, especially in the respiratory, cardiomotor and vasoregulatory centers, are at the origin of the central effects (behavioural disorders, coma, convulsions, respiratory distress and vasoplegia).

In all mammals, poisoning signs are identical for all carbamates. Symptoms onset and severity are dosedependent, first signs usually occurring between 15 to 30 minutes, or less after oral administration [12]. Typically, patients present with tight myosis, sinus bradycardia succeeding a fugitive sinus tachycardia. In severe cases muscarinic-nicotinic and central triad is identified. CNS effects associate behavioural disorders, ataxia, respiratory distress, hypotension, convulsions and coma. In general, death or signs of recovery occur between 1 h and a few hours after intake in relation to the exposure. The usual cause of death is respiratory distress due to several factors such as bronchoconstriction, pulmonary edema, respiratory muscles paralysis, depression of respiratory centres.

Death is usually attributed to asphyxia, but failure of the cardiovascular system cannot be excluded.

Procarbamates exhibit lower toxicity in mammals while retaining insecticidal toxicity similar to that of their parent compound. For example, medial lethal dose (LD50) of thiodicarb (methomyl procarbamate) in rats is of 39 to 136 mg/kg compared to 12 to 48 mg/kg for methomyl) [12].

Elimination

Following digestive uptake of C14-labeled methomyl in rats, pulmonary elimination of carbon dioxide and acetonitrile as well as urinary elimination of other partially identified components were observed in a ratio of 1/2/1. Harvey et al. isolated in urine methomyl, S-methyl-Nhydroxythioacetimidate, sulfoxide and methomyl sulfone [13]. Noda J. reported in a case of human intoxication secondary to ingestion of 2.25 g methomyl, that 6 hours later, the product was present in the blood at a rate of 1.61 ppm (particles per million) and 10.91 ppm in the urine. By the 22nd hour, methomyl was no longer detectable [14].

Currently, many toxicokinetic aspects of carbamates are unknown as certain carbamates have been studied better than others. Differences are related to the routes of administration, type of molecule, its substrate and administered dose. Carbamates share their fast metabolization and elimination [15]. Liver plays a central role given the various enzymatic systems involved. Toxic metabolites and saturable elimination pathways are essential to the toxic risk of each product.

Therapeutic aspects

Two types of treatment are applied in case of carbamate poisoning, symptomatic and antidote with some data on the possible use of oximes.

Symptomatic treatment depends upon absorption pathway (skin, ingestion, and inhalation). Skin or digestive decontamination are necessary (gastric lavage followed by the administration of activated charcoal and mannitol as a purgative).

Orotracheal intubation and assisted ventilation may precede initial management in these patients (possible sedation and administration of anticonvulsants). Oxygen therapy or even respiratory assistance might be necessary prior to atropine administration (risks of serious ventricular arrhythmias on hypoxic myocardium).

Antidote treatment

Atropine (sulphate of atropine) is the anticholinergic of choice: of all the known competitive acetylcholine antagonists in muscarinic receptors, atropine is the one with the greatest affinity and fixation power for the three types of receptors. Moreover, in addition to its predominant action on muscarinic receptors, atropine has a poor effect on nicotinic receptors, and at very high dose, will only induce a partial blockage of the vegetative ganglion and has no effect on the neuromuscular junction. With intramuscular administration, maximum concentration (Cmax) is achieved in 15-30 min due to an irregular resorption in the adult. In case of intravenous injection, the distribution is rapid (distribution half-life of about 1 min) with a decreasing plasma concentration in 8 to 10 min, the atropine level at this time representing only 5% of the administered dose. Endotracheal administration is an option in case of impossible intravenous administration, Cmax being identical to the one registered after intramuscular injection of the same dose [16]. Nevertheless, this pathway is saturable, the adsorption process being limited (dose increase does not lead to a proportional increase in blood concentrations).

Complete, optimal, atropinisation is achieved by administering 0.5 to 2 mg of atropine sulfate every 15 to 20 min. The best criterion for evaluating the completeness of atropinisation are drying up of bronchial and mucosal secretions, tachycardia and mydriasis, which may be unreliable indicators because they are potentially related to nicotinic ganglionic stimulation [7, 10]. Nevertheless, the observation of a pupillary dilatation succeeding a myosis immediately after injection of atropine is a factor to be taken into account even if the pupillary response character appears only partial or temporary. Once complete atropinisation is achieved, repeated boluses or continuous administration (syringe pump) are required in order to maintain therapeutic efficacy.

Oximes (pralidoxime, pralidoxime chloride, pralidoxime mesylate) were introduced by Wilson and Ginsburg in 1955, and qualified as cholinesterase regenerative antidotes widely used in organophosphate poisoning but are known to be less effective or ineffective, and sometimes even partial agonists, and thus are likely to aggravate carbamate poisoning [17].

Lethal dose

In 1979 Liddle et al. reported the case of three patients deceased secondary to carbamate poisoning. The autopsy of one of the patients showed tracheal, bronchial, pulmonary and gastric oedema. They estimated the lethal dose at 12-15 mg/kg [18]. In 1982 Araki et al. reported the case of a collective family suicide of a 31-year-old woman and her 6-year-old child, the autopsies being similar to the previous one, with diffuse oedematous haemorrhagic lesions due to circulatory insufficiency. Lethal doses were estimated to be 55 mg/kg in the mother and 13 mg/kg in the infant [19]. The rapid elimination of carbamates led to an incomplete evaluation of the lethal dose.

Carbamate poisoning is known to be rare and less dangerous than organophosphate poisoning. It is scarcely described in comparison with the latter, but it symptomatology is similar to the organophosphates.

This case presentation tends to prove the extreme initial severity of these intoxications. Depicted signs illustrate the need for optimal rapid medical management which will privilege haematosis (intubation, ventilation) and then atropinisation. Carbamate poisoning is to be suspected in case of rapid installation of severe symptoms similar to organophosphate poisoning.

Conclusions

To our opinion, carbamates exhibit a different toxicity than the organophosphates, with a more brutal, lifethreatening behaviour. Alkyl phosphates and phosphoramides, two classes of organophosphates, need to be metabolized at the hepatic level prior to releasing their active metabolites (for example paraoxon, the active principle of parathion). This delay may induce a latency in symptoms appearance, allowing later medicalization. Immediate intoxication symptoms in case of carbamates are life threatening thus justifying an optimization of the prehospital medical care. In the absence of severe initial complications, a well-conducted symptomatic treatment associated with adequate atropinisation should lead to a rapid regression of disorders allowing an outcome without sequelae. Carbamates should be replaced on the market by procarbamates that retain insecticidal toxicity but are less dangerous for mammals.

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