

## NEW POSSIBILITIES IN THE PHARMACOLOGICAL TREATMENT OF ABNORMAL CONVULSIVE ACTIVITY IN ACUTE ORGANOPHOSPHATE NERVE AGENTS INTOXICATION

<sup>1</sup>Veselin Ivanov, <sup>2</sup>Kameliya Sokolova, <sup>2</sup>Zhivka Tsokeva, <sup>2</sup>Stephan Radev, <sup>3</sup>Borislav Popov

<sup>1</sup>Department of Chemistry and Biochemistry, Faculty of Medicine, Trakia University, 11 Armeiska Str., Stara Zagora - 6000, Bulgaria, veskoasenov@abv.bg

<sup>2</sup>Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Trakia University, 11 Armeiska Str., Stara Zagora - 6000, Bulgaria.

<sup>3</sup>Department of Molecular Biology, Immunology and Medical Genetics, Faculty of Medicine, Trakia University, 11 Armeiska Str., Stara Zagora - 6000, Bulgaria

### ABSTRACT

Organophosphates, used as chemical warfare nerve agents and pesticides are the most toxic chemicals known to date. Despite the number of studies done on the subject matter, intoxication caused by such agents is among the most complicated problems in medicine because of the induced increase in abnormal electrical activity in the brain and the subsequent brain damage. The anticonvulsant and neuro-protective effects of conventional benzodiazepines are limited by their side effects, including sedation, amnesia, withdrawal, and anticonvulsant tolerance. Imidazenil is a proposed drug in the treatment of convulsions in acute cases of poisoning with organophosphate nerve agents and an alternative to the currently used benzodiazepines with an improved safety profile.

**Key words:** imidazenil, neuroprotective effect, organophosphates

Despite the existing ban on chemical weapons (CW), they continue to be a real threat to large numbers of citizens. The population might be impacted via contaminated water supply or mass produced foods in acts of sabotage or acts of terrorism with chemical agents, on public places, such as bus and train or underground stations, airports, public buildings, etc. The nerve agents used as chemical weapons are among the most toxic substances used in chemical warfare. According to their chemical composition they are organophosphates (OP)/carbon-phosphates. They include tabun (GA); sarin (GB); soman (GD) and cyclosarin (GF), forming the so called G-series of chemical warfare nerve agents. The industrial production of these substances started in Germany, during the 1930s, initiated by Dr. Gerhard Schröder's team. The first real warfare agent, belonging to the group, was synthesised in 1936 and was formally approved as a type of chemical weapon to be used by the German Army, under the name of "tabun". The industrial synthesis of sarin, soman and cyclosarin followed in 1938; 1944 and 1949 respectively. G-series chemical warfare agents were used in the WW II, in the Korean and Vietnam wars and in the war between Iran and Iraq. In more recent times chemical warfare nerve agents were used in two terrorist attacks in Japan (Matsomoto, in June 1994 and the one in the Tokyo Underground in March 1995), when sarin, dispersed in the air, caused the death of 12, and acute poisoning of further 5,000 people.

Exposure to organophosphates has a dramatically adverse effect on human health. The mechanism of poisoning consists of the inhibition of acetylcholinesterase (AChE), responsible for the hydrolysis of the neurotransmitter acetylcholine (ACh) in the body. This blockage causes the synaptic levels the mediator to rise, which results in cholinergic hyperactivity, revealed in a wide range of symptoms such as myosis, increased secretion of the exocrine glands, nausea, vomiting, gastrointestinal discomfort. Close attention should be paid to OP-induced increased abnormal bioelectrical (convulsive) brain activity with fascicular muscle twitching, followed by epileptic seizures, which if not treated properly, progress into status epilepticus and can cause irreversible brain damage (1, 2).

In cases of acute OP intoxication, the currently prevalent prevention and therapy schemes include pretreatment with Piridostigmine and application of anticholinergic drugs (Atropine) and

AChE reactivators (oximes). It is true that this combined therapy substantially reduces the morbidity and the death rate of OP intoxication, it is nevertheless, often inefficient in blocking the OP-mediated convulsive activity and the subsequent brain damage they cause (2). It is due to the nature of the neuron-physiological processes, responsible for the existing abnormal electrical brain activity and the neuro-pathological damage caused by exposure to nerve agents. The most commonly presented models of these processes consist of two or three phases. The initial and early occurrence of abnormal electrical brain activity (or of convulsions) is a cholinergic phenomenon, so the anti-cholinergic drugs cease the abnormal bioelectrical brain processes, as a result of which no neuro-pathological damage occurs. If this phase is not interrupted, the second, transitional one occurs, during which the neuron agitation, caused by the change in the electrical activity of the brain, disturbs the proper functioning of other nerve transmission systems as well. It is characterised by increased levels of nerve stimulation inducing amino acids and intensified abnormal electrical brain activity. Cholinergic drugs are less effective in controlling these processes. It is quite possible for slight neuropathological changes to occur. When prolonged epileptic-like activity is evident it marks the beginning of the third, usually non-cholinergic phase. It is characterised by slight neuropathological changes in many brain zones. The excessive calcium influx, due to repetitive depolarisation, induced by the changed electrical brain activity, as well as the prolonged NMDA-receptor stimulation are all viewed as the final cause of the existing neurological pathology (3). At this phase anticholinergic refractory reaction occurs, so benzodiazepins (BDZ) and NMDA-antagonists, co-administered with anticholinergic drugs turn out to be an effective anti-convulsion therapy.

The increased bioelectrical brain activity and convulsions are the main cause of brain damage, so their prevention or, at least, minimisation is the main therapeutic purpose in cases of OP exposure. Early application of anti-convulsion therapy is therefore of vital importance for preventing further distribution of nerve stimulation impulses and the subsequent irreversible brain damage (4). Benzodiazepins are the most frequently used anti-convulsion drugs in cases of acute OP and other nerve agents poisoning.

Adding BDZ, especially Diazepam, to the standard anticholinergic and oxime drugs therapy diminishes the increased bioelectrical brain activity, blocks and completely stops OP-induced convulsions, decreases the neuropathological effects and increases the survival rate among affected victims (5, 6, 7, 8).

The anti-convulsion and neuroprotective effects of BDZ are most intensively produced when applied at an early stage of OP intoxication, before convulsions start, or immediately after exposure to organophosphates. After that a refractory reaction to their effect develops (9, 10, 11, 12, 13).

The described BDZ effects are due to their interaction with specific benzodiazepin receptors, which are an integral part of the post-synaptic  $\gamma$ -aminoacid Cl-complex receptor. In terms of its mechanism of functioning the GABA A receptor is a ligand-binding ion channel, with two binding sites, one for GABA and the other one for benzodiazepins. The combination of BDZ to this receptor potentiates the effects of GABA, whose retention effects on the central neural system are due to the increased chloride influx with a subsequent hyperpolarisation of the neuron cell membrane and inhibition of the nerve transmission process. GABA A consists of a number of sub-units:  $\alpha$ ,  $\beta$  and  $\gamma$  (as well as  $\delta$ ,  $\epsilon$ ,  $\pi$  and  $\theta$ ). They are further divided in six types of  $\alpha$  ( $\alpha 1-6$ ), three types of  $\beta$  ( $\beta 1-3$ ) and three types of  $\gamma$  ( $\gamma 1-3$ ) sub-units. There are also three types of  $\rho$  subunits but presently they are not classified as typical GABA A subunits. The GABA A receptor is formed by 5 subunits, combined in different ways, but is most often presented as consisting of  $2\alpha$ ,  $2\beta$  and  $1\gamma$  ( $\alpha 2\beta 2\gamma$ ) subunits.

The different combinations of particular types of subunits determine the different GABA A receptor localization and properties, and respectively, the differences in their ligands' pharmacological and clinical effects.

BDZs bind to the surface of  $\alpha$  and  $\gamma$  subunits of the GABA A receptor. Different BDZs are attracted to GABAA receptors with different intensity depending on the particular subtypes of GABA A subunits they have. Chemical combination is possible if the  $\alpha$  subunits contain histidine amino acid residue, as it is the case with  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$ , and  $\alpha 5$  containing GABA A receptors. The  $\alpha 4$  and  $\alpha 6$  GABA A receptors contain arginine instead of histidine residues, so BDZ are not attracted to them.

The different subtypes of BDZ have different selectivity, which determines the difference in their pharmacological activity. Relatively new research shows that  $\alpha 1$ -containing GABA A receptors mediate the sedative, amnesic and, to a little degree, the anti-convulsion effect of BDZ, while the anxiolytic and partially, the anti-convulsion activity is mediated by  $\alpha 2$ ,  $\alpha 3$  and  $\alpha 5$  containing GABA A receptors. This revelation drew interest towards the need to find BDZs, which are selective of  $\alpha 2$ ,  $\alpha 3$  and  $\alpha 5$  subunits of the receptor but not affecting  $\alpha 1$  containing receptors, since this would minimize adverse side effects like sedation, amnesia and drug tolerance.

Diazepam, like other “conventional” BDZs, such as Midazolam and Lorazepam act as fully non-selective GABA A receptor agonists, non-specific GABA activity modulators. The lack of receptor selectivity determines the large range of pharmacological activity of these medicines. Along with their anti-convulsion, anxiolytic and hypnotic effect, they also cause dose-related cardiac and respiratory depression, long-term application leads to amnesia, tolerance and drug addiction, which definitely limits their use.

In search for new, selective and less toxic anti-convulsion agents for treating OP intoxication, scientists' attention was drawn to Imidazenil. It belongs to the imidazobenzodiazepines group, together with Midazolam, Flumazenil and Bretazenil. Similarly to typical BDZ, it is a partial selective allosteric modulator of GABA A receptors, which is followed by an increase in chlorine influx, hyperpolarization of neurons and inhibition of nerve transmission (14, 15). Unlike typical BDZs, Imidazenil has low affinity towards  $\alpha 1$  subunit and high affinity to  $\alpha 5$  and most probably to  $\alpha 2$  and 3 subunits (16). That is what determines the pharmacological profile of the drug. Tests on animals revealed its anxiolytic and strong anticonvulsion effect, which are not associated with adverse side effects like sedation, amnesia and myorelaxation, typical for other BDZs. It is proved that the application of Imidazenil on rodents, after exposure to OP, significantly decreases the intensity of convulsions, quickly inhibits the pathological electric brain activity, without affecting coordination and substantially increases the antilethal potential of standard therapy. Its positive therapeutic effects are comparable, even stronger, according to some researchers, that those of Diazepam. Imidazenil proves to have a better safety profile. Adverse side effects, typical for BDZ treatment, such as sedation, drowsiness or thought disorder occur when administering the prescribed therapeutic doses of Diazepam, while with Imidazenil they only occur with doses 5 to ten times higher than the prescribed therapeutic ones (17, 18).

Another big disadvantage, limiting the use of conventional BDZs is the development of tolerance towards their anti-convulsion effect and substance addiction. In different experiments the long-term application anti-convulsion effects of different agonists of benzodiazepin receptors have been compared. It was revealed that laboratory animals develop tolerance towards the anti-convulsion effects of Diazepam and Zolpidem, at multiple application, whereas such tolerance is not observed with Imidazenil. What is more, cross-tolerance has been found to exist between Diazepam and Zolpidem, while Imidazenil does not show cross-tolerance neither with Diazepam, nor with Zolpidem (19, 20). Kadriu et al have come to the same conclusion, comparing the anti-convulsion effects of Diazepam and Imidazenil in a 14 days' experimental application of increasing doses of the medicines, to laboratory animals. The results reveal the decreasing efficacy of Diazepam in controlling the bioelectrical brain activity and convulsions. Imidazenil's efficacy remains constant, which supports the conclusion that there is lack of tolerance towards its anti-convulsion effect (21).

Imidazenil's non-standard/different pharmacological profile makes it potentially successful in treating OP nerve agents intoxication and a better alternative to traditional, currently used medication (22).

**Conclusion:**

Despite the existing international agreement for ban on chemical weapons, the nerve agents are still a threat to humanity. There are still huge numbers of non-disposed chemical weapons, which might be used for terrorist attacks. The disposal of chemical warfare is itself risky because there is no fully safe technology for doing that, so the staff involved in the process and the environment are always at risk. An additional risk to human health is the use of organophosphates in agriculture as crop pesticides. With regard to the severity of the health damage such exposure can cause, it is important for health experts to expand their knowledge of all aspects of OP intoxication treatment, especially of the latest development in existing pharmacological therapy.

**REFERENCES**

1. Solberg Y, M. Belkin , 1997. The role of excitotoxicity in organophosphorous nerve agents central poisoning. *Trends Pharmacol Sci.*,18, 183–185
2. McDonough, JH., TM. Shih, 1997. Neuropharmacological mechanisms of nerve agent induced seizures and neuropathology, *Neurosci. Biobehav. Rev.*, 21, 559-579
3. Shih TM, SM. Duniho, JH. McDonough, 2003. Control of nerve agent-induced seizures is critical for neuroprotection and survival, *Toxicol Appl Pharmacol.*, 188(2), 69-80
4. Marrs TC., 2004. The role of diazepam in the treatment of nerve agent poisoning in a civilian population, *Toxicol Rev.*, 23(3), 145-57
5. McDonough JH, JD, McMonagle , TM, Shih, 2010. Time-dependent reduction in the anticonvulsant effectiveness of diazepam against soman-induced seizures in guinea pigs, *Drug Chem Toxicol.*, 33(3), 279-83
6. McDonough JH, NK. Jaax, RA Crowley, MZ Mays, NE Modorw, 1989. Atropine and/or diazepam protects against soman-induced neural and cardiac pathology, *Fundam Appl Toxicol*, 13, 256-276
7. Shih TM, 1990. Anticonvulsant effects of diazepam and MK-801 in soman poisoning. *Epilepsy Res.*,7, 105–116
8. McDonough JH , TM Shih, 1993. Pharmacological modulation of soman-induced seizures, *Neurosci Biobehav Rev.*, 17, 203-15
9. Lipp JA, 1972. Effect of diazepam upon soman-induced seizure activity and convulsions, *Electroencephalogr Clin Neurophysiol.*, 32, 557–560.
10. Lipp JA, 1973. Effect of benzodiazepine derivatives on soman-induced seizure activity and convulsions in the monkey, *Arch Int Pharmacodyn Ther.*, 202, 244–251
11. Shih T, JH McDonough, I.Koplovitz, 1999. Anticonvulsants for soman-induced seizure activity. *J Biomed Sci.*, 6, 86–96.
12. Lallement G, F. Renault, D.Baubichon, et al., 2000. Compared efficacy of diazepam or avizafone to prevent soman-induced electroencephalographic disturbances and neuropathology in primates: relationship to plasmatic benzodiazepine pharmacokinetics, *Arch Toxicol.*,74, 480–486
13. McDonough JH, LD Zoefel, J. McMonagle, TL. Copeland , CD. Smith, TM Shih., 2000. Anticonvulsant treatment of nerve agent seizures: anticholinergic versus diazepam in soman-intoxicated guinea pigs, *Epilepsy Res.*,38, 1–1
14. Giusti P, I. Ducić , G. Puia R. Arban , A. Walser , A. Guidotti , E. Costa , 1993.. Imidazenil: a new partial positive allosteric modulator of gamma-aminobutyric acid (GABA) action at GABAA receptors, *J Pharmacol Exp Ther.* , 266(2), 1018-28
15. Serra M, CA. Ghiani , C. Motzo , T. Cuccheddu , S. Floris , P. Giusti , G. Biggio, 1994. . Imidazenil, a new partial agonist of benzodiazepine receptors, reverses the inhibitory action of

isoniazid and stress on gamma-aminobutyric acidA receptor function, *J Pharmacol Exp Ther.*, 269(1), 32-8

16. Auta J, F. Impagnatiello , A. Guidotti , E.Costa, 2008. Imidazenil: a low efficacy agonist at alpha1- but high efficacy at alpha-5 GABA receptors fail to show anticonvulsant cross tolerance to diazepam or zolpidem, *Neuropharmacology*, 55, 148-53

17. Rump S, T. Gidynska , E.Galecka , O. Antkowiak, M. Nawrocka, M. Kowalczyk, 2000. Effects of imidazenil, a new benzodiazepine receptor partial agonist, in the treatment of convulsions in organophosphate intoxications, *Neurotoxicity Research.*, 2(1), 17-22.

18. Auta J, E. Costa, J. Davis, A. Guidotti, 2004. Imidazenil: a potent and safe protective agent against diisopropyl fluorophosphate toxicity, *Neuropharmacol*, 46(3), 397-403

19. Auta J, P. Giusti, A. Guidotti, E. Costa , 1994. Imidazenil, a partial positive allosteric modulator of GABA receptors, exhibits low tolerance and dependence liabilities in the rats, *Journal of Pharmacology and Experimental Therapeutics*, 270(3), 1262-9

20. Ghiani CA, M. Serra, C. Motzo, P. Giusti, T. Cuccheddu, ML. Porceddu, G. Biggio, 1994.. Chronic administration of an anticonvulsant dose of imidazenil fails to induce tolerance of GABA receptors in mice, *European Journal of Pharmacology*, 254(3), 299-302.

21. Kadriu B, J.Gocel , J. Larson , A. Guidotti, JM. Davis, MP. Nambiar , J. Auta , 2011.. Absence of tolerance to the anticonvulsant and neuroprotective effects of imidazenil against DFP-induced seizure and neuronal damage, *Neuropharmacology*, 61(8), 1463-9.

22. Wang Y, S. Oguntayo, Y. Wei, E. Elisa, A. Brown, N. Jensen, J.Auta , A.Guidotti , . BP. Doctor, MP. Nambiar, 2012 Neuroprotective effects of imidazenil against chemical warfare nerve agent soman toxicity in guinea pigs, *Neurotoxicology*, 33, (2), 169–177