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Ivermectin effects on motor coordination and contractions of isolated rat diaphragm

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ABSTRACT

Ivermectin, the antiparasitic drug from the macrocyclic lactones class raises attention due to its high efficiency against nematodes and arthropods and very specific toxic and side effects that it may produce in host. Dominant clinical symptoms of adverse effects and toxicity of ivermectin in animals are tremor, ataxia, CNS depression and coma which often results in mortality. In our study increasing intravenous doses of ivermectin, (6 or more times higher than therapeutic dose: 1.25, 2.5, 3.75, 5.0, 6.25 and 7.5 mg/kg), caused dose-dependent disturbance of motor coordination in treated rats. The median effective dose (ED50) that was able to impair the rota-rod performance in rats treated 3 min before testing was 2.52 mg/kg. This effect weakens over time, while in the rats treated 60 min before the rota-rod test, ED50 of ivermectin was 4.21 mg/kg. Whereas, all tested doses of ivermectin did not cause any other clinical symptoms of toxicity. Ivermectin has no effect on the contractions of isolated diaphragm caused by the EFS, which effectively blocked mecamylamine (100 μM) and pancuronium (1 and 2 μM). Effect on motor coordination is the first detectable clinical symptom of ivermectin toxicity and apparently is a result of its central effects.

1. Introduction

Ivermectin is a macrocyclic lactone, member of the avermectins family of compounds derived from the fermentation byproducts of the fungi *Streptomyces avermitilis*. In veterinary medicine, ivermectin is indicated for the treatment of infections with gastrointestinal and respiratory nematodes and arthropods in domestic and wild animals (Adams, 2001). In human medicine ivermectin is the drug of choice for the treatment of lymphatic filariasis and onchocerciasis (Brunton et al., 2005; Katzung, 2006). Besides the high efficiency and wide use in the treatment of parasitic infections, ivermectin and other avermectins have raised considerable attention because of the specific mechanism of antiparasitic action and very unusual side effects in host. The target of its antiparasitic action is ivermectin-sensitive glutamate gated Cl^- channel receptor (GluClR) that exists only in a number of invertebrates and GABA receptor. GluClR is apparently unique to the invertebrate phyla and plays vital roles in the functioning of pharynx, thus accounting for the selective toxicity of these drugs. In contrast to the vertebrates where GABA primarily acts at synapses of the central nervous system (GABA mediates the inhibitory actions of local interneurons in the brain and also mediate presynaptic inhibition within the spinal cord), in invertebrates it acts at neuromuscular synapses. Specifically in nematodes, GABA acts to relax the body muscles by opening chloride channels. However, invertebrate GABA receptors do not readily fit into the mammalian subclassification ($GABA_A$, $GABA_B$, $GABA_C$-subtypes), while many of them are similar to the mammalian $GABA_A$-receptor subtype (Holden-Dye et al., 1989).

Ivermectin and other avermectins exhibit side effects that are mainly associated with GABA-ergic neurotransmission. Dominant clinical symptoms of adverse effects of ivermectin in dogs (Lovell, 1990; Hopper et al., 2002), sheep (Bourke, 1995), pigs (Sanford et al., 1988), horses (Campbell and Benz, 1984; Swor et al., 2009) and other animal species are tremor, ataxia, CNS depression and coma, which often results in mortality. It is not possible to find literature data about ivermectin neurotoxicity in humans, mostly treated against filariasis. This can be explained by a high efficacy of p-glycoprotein, transmembrane protein highly conserved in human population (Lankas and Gordon, 1989), which plays a central role in limiting drug uptake into the brain (Edwards, 2003). In domestic animals, the depressive effect of ivermectin is very common, and may include more than one mechanism.

The mechanisms of action of ivermectin and its analogues have been investigated in several members of this family. The effects of ivermectin on the mammalian $GABA_A$ receptor include potentiation of GABA-gated currents (Dawson et al., 2000; Krusek and Zemkova, 1994) as well as direct, reversible receptor activation (Dawson et al., 2000; Adelsberger et al., 2000). There is also an

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opinion that avermectins act at sites recognized by benzodiazepines on GABA-receptor ionophore complex of chloride channel (Pong et al., 1982). This is confirmed by some characteristics of CNS depression caused by ivermectin that are very similar to certain clinical effects of benzodiazepines. In addition, ivermectin has been known to displace [3H]strychnine in radiolabeled binding studies (Graham et al., 1982) and shows important anticonvulsive properties in strychnine-induced convulsions (Trailović and Varagić, 2007), implying that it may exert some effect on the glycine receptor (GlyR). Finally, ivermectin has been shown to potentiate acetylcholine-mediated responses in the recombinantly expressed neuronal α7 nicotinic acetylcholine receptor (nAChR) (Krause et al., 1998), and thus is an example of a positive allosteric effector that modifies the pharmacological profile of the neuronal α7 nAChR. Neuronal nAChRs are present at presynaptic terminals of other transmitter systems, where they modulate transmitter release. The key subtype mediating these effects is the α7 nAChR, while other subtypes play a role as well. Together these receptors at presynaptic location influence the release of glutamate, GABA, norepinephrine and dopamine among others (Sharma and Vijayaraghavan, 2008). If we used the analogy between the described effects of ivermectin and some benzodiazepines, we can assume that ivermectin like midazolam (Yamamoto et al., 2007) is able to increases the release of GABA via presynaptic α7 nAChR. However, this can be additional depressive mechanism of ivermectin action in the CNS, together with the already well-known agonistic effect of $GABA_A$ receptor.

Considering all this, it was particularly interesting to investigate whether ivermectin affect the rota-rod performance in rats at doses that are not able to cause clinically apparent general depression of CNS. Selection of ivermectin doses we used in this study was carried out based on available literature data about the experimental anticonvulsive doses and the doses of ivermectin able to produce locomotor disorders in the some animal species (Dawson et al., 2000; Davis et al., 1999). Rota-rod test is considered primarily predictive of the anxiolytic, sedative and ataxic influence on CNS of the different drugs. Dunham and Miya (1957) suggested that skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of either mice or rats to remain on a rota-rod. Thereafter the rota-rod test is largely used to evaluate neurological deficits in rodents. According to Massaquoi and Hallett (1998), the loss of motor coordination is characteristic of many neurological disorders, being a pharmacological effect easily detected in cases of intoxication. Rota-rod performance is a good index for neurological deficit because balancing on the rotating rod requires a variety of proprioceptive, vestibular, and fine-tuned motor abilities. This task has been used successfully for detecting acute drug-induced changes in motor coordination as well as neurotoxicity produced by drugs and developmental behavioral abnormalities (Laffan et al., 1989; Jones and Roberts, 1968). Therefore, by using this test we expected to get informations about the values of the ivermectin doses capable to cause first symptom of neurotoxicity, as well as determined whether this effect is dose-dependent and reversible.

It was proven that ivermectin shows certain effects on some subtype of neuronal nicotinic α7 AChRs (Krause et al., 1998), therefore using this analogy we examined the possibility that ivermectin affects the function of nicotinic receptors in the neuromuscular end-plate. To investigate that possibility we have used the contractions of isolated rat diaphragm caused by electric field stimulation (EFS). The testing of contractions of diaphragm preparation is considered as the model to investigate the effect of different substances (Holmstedt, 1959) at the neuromuscular junction (Drazen et al., 1983; Burd and Ferry, 1987; Gayan-Ramirez et al., 1994). The diaphragm has only skeletal muscle and it must have an impulse delivered to contract. The diaphragm receives its motor innervation via the phrenic nerve, with separate branches innervating the crural and costal regions (Gordon and Richmond, 1990). The literature on the topography of phrenic innervation is concerned with three areas: spinal cord organization, nerve root origins, and the projection pattern of phrenic nerve branches. Stimulation of the phrenic nerve is a classic method for testing the contractions of the isolated diaphragm. However, this preparation is unsuitable for larger species or muscle strips from biopsies where no nerve is available for stimulation. An alternative technique is the indirect electrical field stimulation (EFS) of muscles containing intramuscular nerve branches only (Wolthuis et al., 1981). EFS is used to release of neurotransmitters and provoke contractions of diaphragm tissues. While, always the same size of diaphragm strips provides consistency of results. Therefore, it was important for us to investigate whether ivermectin affect the ACh release and its binding to the muscle nicotinic AChRs.

All experiments have been done in order to elucidate the mechanism of the toxic effects of ivermectin in animals.

2. Materials and methods

2.1. Animals

White male Wistar rats, weighing 150–200 g were housed under standard conditions for laboratory animals in groups of five with controlled 12-h light/dark cycle, temperature of 21–24 °C, and "ad libitum" access to standard diet and water. All procedures in the study conformed to EEC Directive 86/609 and were approved by the Ethic Committee of the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine University of Belgrade. At the end of the experiments all the rats were humanely euthanized by the overdose of pentobarbitone in accordance with the Home Office Code of Practice (1997).

2.2. Drugs and method of administration

Sigma Chemical Co. (St. Louis, MO, USA) supplied ivermectin, mecamylamine, and pancuronium. Ivermectin was dissolved in propylene glycol + glycerol formol (60:40 v/v) for intravenous administration.

Ivermectin was administered by slow intravenous injection (into lateral tail vein), using an intravenous cannula Optiva TM 2 (Johnson and Johnson, Arlington, TX, USA). Injected volume did not excess 0.1 ml/100 g body weight.

2.3. Procedures

2.3.1. Rota-rod performance

The rota-rod test has been used to assess motor coordination and balance alterations in rodents (Jones and Roberts, 1968). This test measured the ability of the rat to preserve balance holding itself on the rotating rod (Ugo Basile 7700, Italy). Before the investigation, animals were trained for 2 days in order to remain for 180 s on the rod rotating at constant speed of 8 rpm. Three trials per day during two days were enough for the animals to learn this task. On the day of the test only rats able to stay balanced on rotating rod for 180 s were selected. The test was performed on well-trained 84 rats divided into 14 equal groups ($n = 6$). The effect of increasing doses of ivermectin on the integrity of motor coordination was assessed based on the ability of rats to stay on rotated rod for 3 min without falling and 3 or 60 min after drug administration. The increasing doses of ivermectin 1.25, 2.5, 3.75, 5.0, 6.25 and 7.5 mg/kg or solvent 0.1 ml/100 g for control rats, were injected intravenous 3 min (seven groups of rats) or 60 min (another seven