Molecular & Biochemical Parasitology 188 (2013) 79–86

Contents lists available at SciVerse ScienceDirect

Molecular & Biochemical Parasitology

Derquantel and abamectin: Effects and interactions on isolated tissues of *Ascaris suum*

Sreekanth Puttachary[a], Sasa M. Trailovic[a,1], Alan P. Robertson[a,*], David P. Thompson[b], Debra J. Woods[b], Richard J. Martin[a]

[a] *Department of Biomedical Sciences, Iowa State University, Ames, IA 50011, USA*
[b] *Zoetis, 333 Portage Street, Kalamazoo, MI, USA*

ARTICLE INFO

Article history:
Received 29 October 2012
Received in revised form 5 February 2013
Accepted 15 February 2013
Available online 21 March 2013

Keywords:
Abamectin
Derquantel
Combination
Interaction
nAChRs
GluCls

ABSTRACT

Startect® is a novel anthelmintic combination of derquantel and abamectin. It is hypothesized that derquantel and abamectin interact pharmacologically. We investigated the effects of derquantel, abamectin and their combination on somatic muscle nicotinic acetylcholine receptors and pharyngeal muscle glutamate gated chloride receptor channels of *Ascaris suum*. We used muscle-strips to test the effects of abamectin, derquantel, and abamectin + derquantel together on the contraction responses to different concentrations of acetylcholine. We found that abamectin reduced the response to acetylcholine, as did derquantel. In combination (abamectin + derquantel), inhibition of the higher acetylcholine concentration response was greater than the predicted additive effect. A two-micropipette current-clamp technique was used to study electrophysiological effects of the anthelmintics on: (1) acetylcholine responses in somatic muscle and; (2) on L-glutamate responses in pharyngeal preparations. On somatic muscle, derquantel (0.1–30 μM) produced a potent (IC_{50} 0.22, CI 0.18–0.28 μM) reversible antagonism of acetylcholine depolarizations. Abamectin (0.3 μM) produced a slow onset inhibition of acetylcholine depolarizations. We compared effects of abamectin and derquantel on muscle preparations pretreated for 30 min with these drugs. The effect of the combination was significantly greater than the predicted additive effect of both drugs at higher acetylcholine concentrations. On the pharynx, application of derquantel produced no significant effect by itself or on responses to abamectin and L-glutamate. Abamectin increased the input conductance of the pharynx (EC_{50} 0.42, CI 0.13–1.36 μM). Our study demonstrates that abamectin and derquantel interact at nicotinic acetylcholine receptors on the somatic muscle and suggested synergism can occur.

1. Introduction

Nematode parasites cause severe problems for humans and animals. Globally, >1 billion people are infected with ascariasis, hook worms and whipworms; soil transmitted gastro-intestinal (GI) nematodes. These infections are endemic in a majority of tropical countries [1,2]. Similar GI nematodes also infect most domestic animals. Nematode infections in both humans and livestock result in debility, reduced productivity, severe economic losses and contribute to poverty [3,4].

In the absence of effective vaccines and sanitation, anthelmintic drugs are used for treatment and prophylaxis. Unfortunately, the regular use of anthelmintics has resulted in the emergence of anthelmintic resistance in domestic animals with similar concerns developing for humans. In Australia [5–7], anthelmintic resistance threatens the economics of the entire sheep industry. In humans, helminth isolates (hookworm and schistosmiasis) have been described which are resistant to anthelmintic treatment [8–11]. The limited number of anthelmintics available for therapy, coupled with the development of resistance in parasites poses a serious threat to livestock and are a concern for human health [4,12].

A majority of anthelmintics exert their effect selectively on membrane ion-channels of nematode parasites. Nicotinic acetylcholine receptors (nAChRs) are present on nematode somatic muscles and nerves. Anthelmintics that act as selective agonists at muscle nAChRs produce spastic paralysis, while selective antagonists produce flaccid paralysis. The nAChR agonists include: the imidazothiazoles (levamisole); the tetrahydropyrimidines (pyrantel and oxantel) [13] and the amino-acetonitrile derivatives (monepantel) [14]. The nAChR antagonists include the spiroindoles (derquantel, Fig. 1) [15]. Inhibitory glutamate gated chloride

* Corresponding author. Tel.: +1 515 294 1212; fax: +1 515 294 2315.
E-mail address: alanr@iastate.edu (A.P. Robertson).
[1] Current address: Department of Pharmacology & Toxicology, Faculty of Veterinary Medicine, Belgrade, Republic of Serbia.

0166-6851/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.molbiopara.2013.02.004

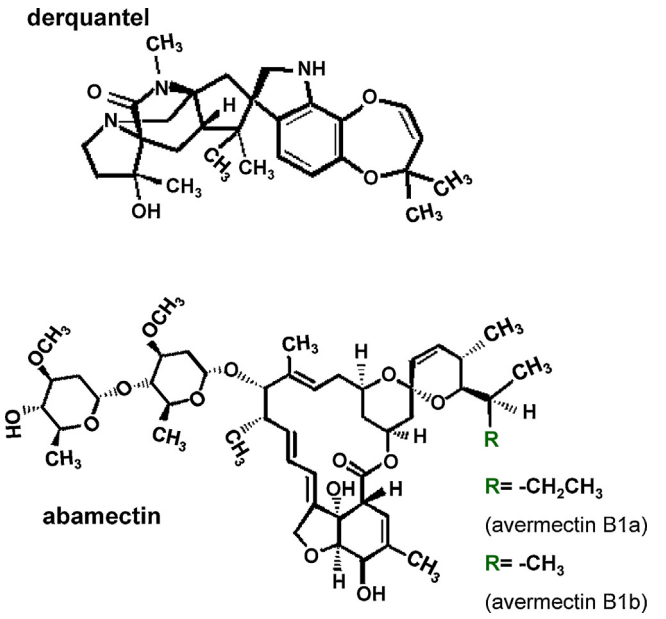

Fig. 1. Structures of derquantel and abamectin. Abamectin is a mixture containing more than 80% avermectin B1a and less than 20% avermectin B1b. Avermectin B1a differs from avermectin B1b by a functional group at the 'R' position.

channels (GluCls) are present on nematode pharyngeal muscle [16] and are also widely distributed on nematode neurons. The avermectins (ivermectin and abamectin, Fig. 1) and milbemycins (moxidectin and milbemycin) increase opening of GluCls [16,17], inhibiting pharyngeal pumping and feeding [18].

If control of parasitic nematodes relies on only a single class of anthelmintic drug, the selection pressure for resistance is strong [19]. However, if a combination of anthelmintic drugs is used from different drug classes, the development of resistance is predicted to be slower because simultaneous development of resistance for two classes of anthelmintic is required [20–24]. The combination of two or more anthelmintics also has the potential of producing additive or synergistic effects, increasing the efficacy of the combination.

In this study, we used isolated tissues from *A. suum* to study effects of derquantel and abamectin. We used somatic muscle flaps for contraction assays. We used somatic muscle flaps and pharyngeal muscles for electrophysiological assays. We studied the effects of derquantel alone, abamectin alone and both in combination; we found that the effects of derquantel and abamectin combined and found that synergism can occur on the nAChRs of the muscle. Derquantel had no effect on the pharynx.

2. Materials and methods

Adult *A. suum* were collected weekly from the JBS packing plant at Marshalltown, Iowa. Worms were maintained in Locke's solution [composition (mM): NaCl 155, KCl 5, $CaCl_2$ 2, $NaHCO_3$ 1.5 and glucose 5, at a temperature of 32 °C. The Locke's solution was changed twice daily and each batch of worms was used within 4 days of collection.

2.1. Muscle-flap for contraction

We prepared 1 cm muscle body flaps by dissecting the anterior part of the worm, 2–3 cm caudal to the head. Each flap was monitored isometrically by attaching a force transducer in an experimental bath maintained at 37 °C containing 10 ml *Ascaris* Perienteric Fluid Ringer/APF Ringer (mM): NaCl, 23; Na-acetate, 110; KCl, 24; $CaCl_2$, 6; $MgCl_2$ 5; glucose, 11; HEPES, 5; pH 7.6 with NaOH and 0.1% DMSO and bubbled with nitrogen. After dissection, the preparations were allowed to equilibrate for 15 min under an initial tension of 0.5 g. Different concentrations of acetylcholine were then added to the preparation and the maximum contraction observed before washing and subsequent application of the next concentration of acetylcholine. The responses for each concentration were expressed as a % of the maximum tension produced by each individual flap preparation. The effects of abamectin, and derquantel on control acetylcholine dose–response plots were determined. Contraction was monitored on a PC using a MacLab interface. The system allows for recording, displaying and analysis of experimental data. Sigmoid dose–response curves for each individual flap preparation at each concentration of antagonist were described by the Hill equation. The contraction responses were normalized by dividing each response by the mean of all of the responses of the 100 μM acetylcholine responses (n = 15 preparations).

2.2. Muscle flap for current-clamp recording

We also prepared the 1 cm muscle body flaps for electrophysiology by dissecting the anterior part of the worm, 2–3 cm caudal to the head which were then pinned onto Sylgard™ contained in lined double jacketed bath chamber maintained at 35 °C by an inner circulation of warm water (Fisher scientific Isotemp 3016H, PA, USA). The preparation was continuously perfused, with APF-Ringer, composition (mM): NaCl 23, Na-acetate 110, KCl 24, $CaCl_2$ 6, $MgCl_2$ 5, glucose 11, and HEPES 5; 0.1% DMSO; NaOH or acetic acid was used to adjust the pH to 7.6.

The incoming perfusate was pre-warmed to 35 °C with an in-line heating system (SH 27B Warner instruments, CT, USA) before application. The rate of perfusion was 3.5–4 ml min^{-1} through a 20 gauge needle placed directly above the muscle bag recorded from. Test compounds were dissolved in APF-Ringer and applied as described in the results. A two-microelectrode current-clamp technique was employed to examine the electrophysiological effects in the bag region of somatic muscle. We used 3 M potassium acetate in the micropipettes which had resistances of 20–30 MΩ. The recordings were made by impaling the bag region of somatic muscle with two microelectrodes, namely current-injecting (I) and voltage-recording electrodes (V). The step current was −40 nA was injected for 500 ms at 0.3 Hz. All experiments were performed using an Axoclamp 2A amplifier, a 1320A Digidata interface and Clampex 9 software (Molecular Devices, CA, USA). All data were displayed and analyzed on a PC based desktop computer. Our somatic muscle preparations had resting membrane potentials greater than −25 mV and the resting input conductances less than 4 μS.

2.3. The pharyngeal muscle preparation

The pharynx of *Ascaris* is a large muscular tube amenable to electrophysiological study. The cuticle and the muscle in the head region were dissected out to expose the pharynx. The beginning of the intestine was pinned to the muscle and the cuticle to secure the pharynx for recording. We increased the stability of the preparation by using calcium-free APF Ringer to limit contraction and by lowering the temperature of the incoming perfusate to 28 °C. A microperfusion needle with a flow rate of 3.5–4 ml min^{-1} was used for perfusion of the pharynx. The recordings were made by impaling at the posterior region of pharynx with two microelectrodes, namely current-injecting (I) and voltage-recording electrodes (V). The step current of −1000 nA was injected for 500 ms at 0.3 Hz. Our pharyngeal muscle preparations had resting membrane potentials greater than −15 mV and the resting conductances less than 250 μS. Test compounds were dissolved in the calcium free