The fumarate reductase system as a site of anthelmintic attack in *Ascaris suum*

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Various benzimidazole compounds have been shown to be highly effective as inhibitors (up to 50% reduction of activity) in vitro of the helminth-specific enzyme, fumarate reductase, of *Ascaris suum*. Anthelmintically active and inactive benzimidazoles were similarly effective as inhibitors of enzyme activity. Albendazole-induced inhibition of fumarate reductase was not observed when the enzyme was preincubated with NADH.

The parasite-specific enzyme fumarate reductase catalyses an important fermentative reaction in many helminths. Fumarate reductase has been shown to be susceptible to inhibition by several anthelmintic benzimidazole compounds (e.g. Prichard, 1970, 1973; Bryant, 1983) and, therefore, it has been proposed that fumarate reductase is a possible site of chemotherapeutic attack.

A range of benzimidazole compounds and their anthelmintically inactive metabolites were investigated for their effect on fumarate reductase activity in vitro.

**Materials and Methods**

**Preparation of fumarate reductase extracts**

The intestines of freshly collected *Ascaris suum* (obtained from a local abattoir) were homogenized in 10-mM KH$_2$PO$_4$/K$_2$HPO$_4$ buffer pH 7 containing 1-mM MgSO$_4$ and the homogenate centrifuged at 3000 g for 10 min. The resulting supernatant was centrifuged at 200 000 g for 1 h and the supernatant discarded. The pellet was resuspended in the above buffer and fumarate reductase activity solubilized by incubation in a sonic bath for 30 min. This suspension was centrifuged at 3000 g for 30 min and the supernatant assayed for fumarate reductase activity.

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Measurement of fumarate reductase activity

The rate of NADH oxidation at A_{340} following addition of sodium fumarate was measured as described by Prichard (1973). Endogenous rates of NADH oxidation have been subtracted from the presented results.

In the experiments measuring inhibition of fumarate reductase by benzimidazole compounds, samples were preincubated with drug (or control buffer) for 0.5 h at 37°C before assay. The percentage inhibition achieved was fairly consistent between batches of worms, despite differences in absolute reaction rates. Protein determinations were made according to the method of Lowry et al. (1951).

Preparation of benzimidazole solutions

Benzimidazole solutions were prepared in ethanol (final concn. of 100 µg ml⁻¹) because of their low aqueous solubility, and diluted in homogenizing buffer immediately before use. The residual amount of ethanol was added to the control buffers.

Results

The anthelmintically active benzimidazole, albendazole sulphoxide, was shown to inhibit the fumarate reductase activity of A. suum (Table 1). Maximal inhibition was achieved when extracts of fumarate reductase were preincubated with albendazole sulphoxide at concentrations greater than 0.05 µg ml⁻¹.

A range of benzimidazoles and their host metabolites (at a final concentration of 0.1 µg ml⁻¹) were investigated for their effect on the fumarate reductase system (Table 2). As these benzimidazole compounds mimic the antimicrotubular effects of colchicine on A. suum in vitro (Barrowman et al., 1984) preincubation of enzyme extracts

Table 1. Inhibition of fumarate reductase activity in A. suum by albendazole sulphoxide

<table>
<thead>
<tr>
<th>Albendazole sulphoxide concn. (µg ml⁻¹)</th>
<th>% Remaining fumarate reductase activity</th>
</tr>
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<tbody>
<tr>
<td>0 (Control)</td>
<td>100</td>
</tr>
<tr>
<td>0.001 (4 × 10⁻⁹ M)</td>
<td>101</td>
</tr>
<tr>
<td>0.005 (2 × 10⁻⁸ M)</td>
<td>96</td>
</tr>
<tr>
<td>0.01 (4 × 10⁻⁸ M)</td>
<td>85</td>
</tr>
<tr>
<td>0.5 (2 × 10⁻⁷ M)</td>
<td>76</td>
</tr>
<tr>
<td>0.1 (4 × 10⁻⁷ M)</td>
<td>62</td>
</tr>
<tr>
<td>1.0 (4 × 10⁻⁶ M)</td>
<td>61</td>
</tr>
</tbody>
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