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Short communication

Binding of serum albumin to the anthelmintic drugs albendazole, triclabendazole and their sulphoxides

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ABSTRACT

The binding of drugs to plasma proteins – especially serum albumin – is an important factor in controlling the availability and distribution of these drugs. In this study we have investigated the binding of two drugs commonly used to treat liver fluke infections, albendazole (ABZ) and triclabendazole (TCBZ), and their sulphoxide metabolites to bovine serum albumin (BSA). Both ABZ and TCBZ caused shifts in the mobility of BSA in native gel electrophoresis. No such changes were observed with the sulphoxides under identical conditions. The drugs, and their sulphoxides, caused quenching of the intrinsic tryptophan fluorescence of BSA, indicating association between the drugs and this protein. Quantification of this quenching suggested a 5–10-fold reduction in affinity of the sulphoxides compared to the parent compounds. These results are discussed in respect to previous work on the pharmacodynamics of these fasciolicides and will inform the design of novel anthelmintics.

1. Introduction

Benzimidazole drugs are commonly used to treat helminth infections of humans and animals. Albendazole (ABZ, Fig. 1) is widely used in the treatment of trematode and nematode infections (de Silva et al., 1997; van den Enden, 2009). The drug of choice for treating infections by the liver fluke (*Fasciola hepatica*) infections in humans and animals is triclabendazole (TCBZ) (Fig. 1) (Fairweather, 2005; Brennan et al., 2007). Both ABZ and TCBZ are active against mature flukes, but only TCBZ is also active against the juvenile form (Bennett and Kohler, 1987; Stitt and Fairweather, 1993, 1994). Both compounds are metabolised by the host to sulphoxide derivatives (Fig. 1) which are more soluble than the parent compounds (Hennessy et al., 1987; Kinabo and Bogan, 1988; Delatour et al., 1990; Alvarez-Bujidos et al., 1993; Virkel et al., 2006). Following administration of the parent compounds, only the sulphoxide, sulphone and various hydroxylated forms were detected in plasma from the systemic circulation (Marriner and Bogan, 1980; Mohammed Ali et al., 1986; Alvarez et al., 2000). Low concentrations of ABZ have been detected in the hepatic portal vein (Alvarez et al., 2000).

Liver fluke infections are of increasing importance in livestock. This has profound effects on agriculture with global losses estimated at $3 billion in 1994 (Boray, 1994). There are also an estimated 2.4–17 million humans infected with the parasite, mostly in the developing world (Mas-Coma et al., 2009a; Robinson and Dalton, 2009). These increased incidences of infection result from two primary factors. Changes in climate, resulting in warmer wetter summers, have favoured the intermediate snail host (*Galba truncatula*) and the emergence of resistance to both ABZ and TCBZ have rendered these drugs less effective as control agents (Moll et al., 2000; Thomas et al., 2000; Alvarez-Sanchez et al., 2006; Brennan et al., 2007; Kenyon et al., 2009; Mas-Coma et al., 2009b). ABZ acts by binding to β-tubulin and disrupting the polymerisation of microtubules (Fetterer, 1986; Lacey and Prichard, 1986; Lubega

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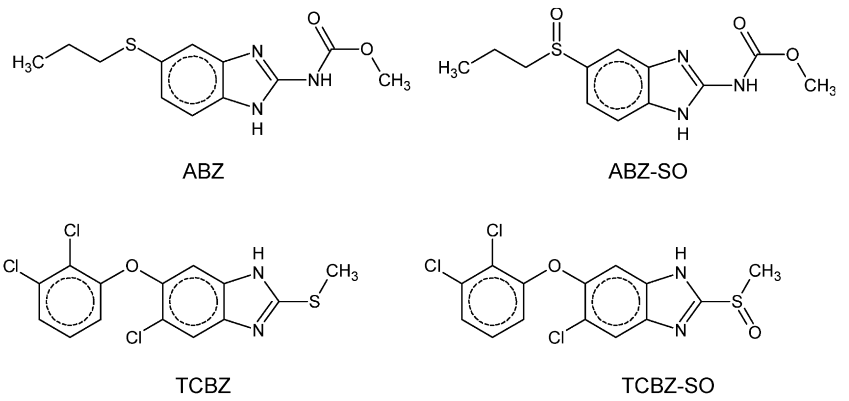

Fig. 1. The structures of the drugs used in this study.

and Prichard, 1991; Oxberry et al., 2001; MacDonald et al., 2004). ABZ-SO also interacts with β-tubulin, although with lower affinity (MacDonald et al., 2004). The precise, molecular mechanism of action of TCBZ is unknown. It has been hypothesised that, like ABZ, it disrupts microtubule formation. This is supported by the observation that processes (including vesicle transport and cell division) which rely on these cytoskeletal elements are disrupted by TCBZ (Stitt and Fairweather, 1994; Robinson et al., 2002; Buchanan et al., 2003; Robinson et al., 2003; Fairweather, 2005; Ryan et al., 2008).

Like many drugs, ABZ, TCBZ and their sulphoxides are carried by the bloodstream in the host where they are exposed to high concentrations of plasma proteins. Serum albumin binds to many non-polar molecules, including some drugs. This binding reduces the plasma concentration of free drug. The free drug concentration is critical in controlling the effects of most drugs (Toutain and Bousquet-Melou, 2002). Thus, understanding a drug's interactions with serum albumin is important in the development of pharmacodynamic models of its action. Consequently, we investigated and quantified interactions of ABZ, ABZ-SO, TCBZ and TCBZ-SO with BSA.

2. Methods

2.1. Materials

Bovine serum albumin (BSA), ABZ, and TCBZ were purchased from Sigma (Poole, UK). Drugs were dissolved initially in DMSO to make 5 mM stocks and then diluted in Buffer Z (50 mM Hepes-OH, 150 mM NaCl, 10% glycerol, 1 mM DTT, pH 7.5). BSA was dissolved in buffer Z.

2.2. Native gel electrophoresis

BSA (10 μM) and drugs (10 and 100 μM) were mixed in a final volume of 10 μl and allowed to equilibrate for 90 min at 22 °C. An equal volume of native gel loading buffer (100 mM Tris, pH 6.8, 24% (v/v) glycerol, 0.02% (w/v) bromophenol blue) was added and the samples resolved by electrophoresis on 15% native polyacrylamide gels made up in 400 mM Tris–HCl pH 8.8. Electrophoresis was carried out at 30 mA (constant current) for 120 min using 25 mM Tris base, 250 mM glycine, pH 8.3 as a running buffer. Gels were stained with Coomassie Brilliant Blue R250 (Sigma) dissolved in 45% (v/v) ethanol/10% (v/v) acetic acid and destained with 7.5% (v/v) acetic acid/5% (v/v) ethanol.

2.3. Fluorescence binding assays

Mixtures of BSA (20 μM) and drugs (5–400 μM) were prepared and incubated at 22 °C for 90 min. The intrinsic tryptophan fluorescence was measured in a Spectra Max Gemini XS fluorimetric platereader. Each reading (excitation wavelength 280 nm; emission wavelength 340 nm) was taken in triplicate to give a mean fluorescence value (in relative fluorescence units, RFU) of F_{raw}. To correct for background and any fluorescence of the drug, triplicate readings of the fluorescence emitted by identical concentrations of drugs in the absence of BSA were recorded ($F_{background}$). The corrected fluorescence readings (F) were calculated as $F = F_{raw} - F_{background}$. The net change in fluorescence (ΔF) was calculated as $\Delta F = |F - F_0|$ where F_0 is the corrected fluorescence at a drug concentration of zero. The error in each value of ΔF was estimated as the sum of the standard deviations in F_{raw}, F_{raw0}, $F_{background}$ and $F_{background0}$ where F_{raw0}, and $F_{background0}$ are the fluorescence measurements of BSA and buffer in the absence of drug. The data were fitted to the equation $\Delta F = \Delta F_{max}[Drug]/(K_{d,app} + [Drug])$ (where ΔF_{max} is the maximum, limiting value of ΔF and $K_{d,app}$ is the apparent dissociation constant for the drug–BSA interaction) by non-linear curve fitting (Marquardt, 1963) as implemented in the program GraphPad Prism (version 3.02) (GraphPad Software, San Diego, USA). All points were weighted equally.

3. Results and discussion

When 10 μM BSA was resolved on a native gel in the presence of 10 μM ABZ, TCBZ, ABZ-SO or TCBZ-SO, no mobility shift was observed (data not shown). However, when the drug concentrations were increased to 100 μM, shifts were observed with ABZ and TCBZ, but not ABZ-SO and TCBZ-SO (Fig. 2). Since mobility on native gels depends mainly on the hydrodynamic volume and charge of the