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Serum albumin and α-1 acid glycoprotein impede the killing of *Schistosoma mansoni* by the tyrosine kinase inhibitor Imatinib

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ABSTRACT

In the search for new drugs and drug targets to treat the flatworm disease schistosomiasis, protein kinases (PKs) have come under particular scrutiny because of their essential roles in developmental and physiological processes in schistosome parasites. In this context the application of the anti-cancer Abl tyrosine kinase (TK) inhibitor Imatinib (Gleevec/Glivec; STI-571) to adult *Schistosoma mansoni* *in vitro* has indicated negative effects on diverse physiological processes including survival.

Motivated by these *in vitro* findings, we performed *in vivo* experiments in rodent models of *S. mansoni* infection. Unexpectedly, Imatinib had no effect on worm burden or egg-production. We found that the blood components serum albumin (SA) and alpha-1 acid glycoprotein (AGP or orosomucoid) negated Imatinib's deleterious effects on adult *S. mansoni* and schistosomula (post-infective larvae) *in vitro*. This negative effect was partially reversed by erythromycin. AGP synthesis can increase as a consequence of inflammatory processes or infection; in addition upon infection AGP levels are 6–8 times higher in mice compared to humans. Therefore, mice and probably other rodents are poor infection models for measuring the effects of Imatinib *in vivo*. Accordingly, we suggest the routine evaluation of the ability of AGP and SA to block *in vitro* anti-schistosomal effects of small molecules like Imatinib prior to laborious and expensive animal experiments.

1. Introduction

Schistosomiasis is caused by a number of schistosome species, which belong to the class Trematoda within the phylum platyhelminthes. According to recent WHO data, about 780 million people are at risk of infection, and more than 240 million patients require treatment each year (Ross et al., 2002; Steinmann et al., 2006; King, 2010; World Health Organization, 2013). Besides humans, infection in farm and wild animals induces similar pathological consequences (De Bont and Vercruysse, 1998; Wang et al., 2006; Wu et al., 2010). Therefore, schistosomiasis represents not only a medical but also a serious socio-economic problem, which affects both developing and newly industrializing countries (Huang and Manderson, 2005; King, 2010; McManus et al., 2010; Chen, 2014).

Three drugs have been available to treat schistosomiasis, metrifonate (against *Schistosoma haematobium*; no longer commercially available), oxamniquine (active only against *Schistosoma mansoni*; restricted availability), and praziquantel (PZQ). The latter is the only drug effective against all important schistosome species and consequently, as recommended by the WHO, is the drug of choice applied in preventive chemotherapy programs worldwide (Harder, 2002; Magnussen, 2003; Fenwick et al., 2006; Mathers et al., 2007; Stothard et al., 2009; Danso-Appiah et al., 2013). However, PZQ has notable failings as a drug: (i) it mainly targets the adult worm whereas the immature forms between 7 and 28 days post-infection (p.i.) are less susceptible; (ii) complete cure is rarely achieved in the single 40 mg/kg recommended dose for MDA; (iii) this drug it is not free of adverse effects (Doenhoff et al., 2008; Caffrey et al., 2009) and (iv) with the increasingly widespread and regular application, there is justified fear of emerging resistance. Laboratory experiments have shown that reduced susceptibility against PZQ is inducible upon selection pressure (Doenhoff et al., 2008; Sabra and Botros, 2008; Pica-Mattoccia et al., 2009). Clinically relevant proof of resistance has not been reported yet, however, results of

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field studies indicate decreased PZQ efficacy (Ismail et al., 1999; Black et al., 2009; Melman et al., 2009). Due to the availability of genome data for the three important schistosome species infecting humans (Berriman et al., 2009; Schistosoma japonicum Genome Sequencing and Functional Analysis Consortium, 2009; Protasio et al., 2012; Young et al., 2012), the existence of multidrug-transporters has been confirmed and initial characterizations demonstrate that a P-glycoprotein efflux pump and multidrug resistance-associated proteins of *S. mansoni* are responsive to PZQ (James et al., 2009; Kasinathan and Greenberg, 2012; Greenberg, 2013).

Due to the lack of a vaccine and limited drug availability, the WHO ranks schistosomiasis next to malaria and tuberculosis in importance as a tropical disease for which novel treatment strategies are urgently needed (Steinmann et al., 2006; Montresor et al., 2012; World Health Organization, 2013). Many research initiatives are underway, and new targets have come into focus (Caffrey, 2007; Caffrey and Selzer, 2012; Geary, 2012; Huang et al., 2012; Prichard et al., 2012). Among these, the TKs that have been extensively studied during the last decade for their pleiotropic functions in development, growth including mitosis, reproduction, tissue integrity, and survival (Swierczewski and Davies, 2010; Dissous and Grevelding, 2011; Buro et al., 2013; de Saram et al., 2013; Dissous et al., 2013; Andrade et al., 2014). The biological functions of these TKs and their roles as presumptive candidates for targeting were elucidated by *in vitro*-culture of adults and/or larval stages with small molecule inhibitors and/or RNAi.

Among the TKs studied, the *S. mansoni* orthologs of the Abelson murine leukemia (Abl) TKs, SmAbl1 and SmAbl2, have been characterized in particular detail. By *in situ* hybridization using adults, transcripts for SmAbl1 and SmAbl2 have been detected in the gonads, the area surrounding the ootype, and the parenchyma and/or the gastrodermis indicating their involvement in reproduction and other physiological processes (Beckmann and Grevelding, 2010). Comparative sequence analyses have shown that these SmTKs possess the majority of amino acid residues necessary for human Abl-kinase to bind to Imatinib (Nagar et al., 2002; Beckmann and Grevelding, 2010). Imatinib is a small-molecule inhibitor marketed as Glivec (Gleevec/STI-571), it acts as a competitive antagonist of the adenosine triphosphate (ATP) binding site of Abl-TKs, and is used to treat chronic myelogenous leukemia and other human cancers (Manley et al., 2002; Larson et al., 2008). Biochemical studies have confirmed that both schistosome Abl-TKs are targets for Imatinib (Beckmann et al., 2011; Buro et al., 2014). Studies with adult schistosomes *in vitro* demonstrated dose- and time-dependent effects of Imatinib, including body swellings, defects in locomotion, reduced pairing stability and viability. Microscopic analyses revealed degenerative changes within the gonads such as disordered apoptotic oogonia and smaller testes with defective sperm differentiation. The most remarkable effect, however, was the degradation of the gastrodermis that caused the death of the parasites (Beckmann and Grevelding, 2010).

To further analyze the potential of Imatinib as an anti-schistosomal therapy, we employed mouse and hamster infection models as well as *in vitro* studies to investigate the effect of Imatinib *in vivo* and *in vitro* with a special focus on specific host-blood components.

2. Material and methods

2.1. Parasite stocks

Adult and larval schistosome stages originated from a Liberian (Grevelding, 1995) and a Puerto Rican (Abdulla et al., 2009) isolate of *Schistosoma mansoni*, respectively. Both were maintained in snails (*Biomphalaria glabrata*) and Syrian hamsters (*Mesocricetus auratus*). Cercariae were obtained from snails after 30 days of infection. Adult parasites were obtained by hepato-portal perfusion at 42–49 days p.i. as described before (Grevelding, 1995; Abdulla et al., 2009). Experiments were approved by the regional council Giessen, Germany (V54-19 c 20/15 c GI 18/10 and V54-19 c 20/15 (1) GI 18/10 – Nr.75/2009). All animal procedures performed at the UCSF, USA, were done in accordance with protocols approved by the UCSF Institutional Animal Care and Use Committee (IACUC) as required by the Federal Animal Welfare Act and the National Institutes of Health Public Health Service Policy on Humane Care and Use of Laboratory Animals (http://grants.nih.gov/grants/olaw/references/phspol.htm).

2.2. Infection and treatment of rodents

For these experiments 12 mice (NMRI) and 12 hamsters (*Mesocrietus auratus*) were used in Giessen (Germany), and 6 hamsters (*Mesocrietus auratus*) at the UCSF (USA). In Giessen, infections with schistosomes were performed by the paddling method as described before using 2000 cercariae per hamster and 800 per mouse (Grevelding, 1995). At the UCSF, 4–6 week old, female hamsters were infected sub-cutaneously with 800 cercariae of a Puerto Rican isolate of *S. mansoni* (Abdulla et al., 2009).

Previously obtained data indicated killing of adult parasites within 1-2 days using 100 μM, or within 4–7 days using 1–10 μM *in vitro* (Beckmann and Grevelding, 2010; Dissous and Grevelding, 2011). Experiments in the mouse model have shown an elimination half-life of 1.3 h for Imatinib in mice and tissue/plasma concentration ratios of Imatinib of about 3.76 ± 1.09, and 12.0 ± 6.3 μg/ml after applications of 25 mg/kg or 50 mg/kg (Teoh et al., 2010). For liver, tissue/plasma concentration ratios between 19.2 ± 13.3 μg/g, and 61.4 ± 40.1 μg/g were determined after multiple dosages of 25 mg/kg or 50 mg/kg, respectively (Teoh et al., 2010). With respect to these data and pharmacokinetic considerations concerning the application of a drug for humans in rodents (Fabbro et al., 2002; Kretz et al., 2004; Löscher et al., 2006) different dosages of 20–100 mg/kg body weight were applied. From days 40 p.i. or 34 p.i. on, Imatinib (dissolved in 0.9% NaCl) was given at a daily basis by gavage over 4-days or 10-days periods, respectively, which was conducted by a veterinarian. The constitution of the animals was checked daily. One day after the final treatment, the animals were euthanized and the parasites recovered by perfusion.

2.3. In vitro culture of adult schistosomes

After perfusion, adult schistosomes were washed three times with M199 medium before being cultured in the same medium (Gibco; including glucose, sodium bicarbonate, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) supplemented with an antibiotic/antimycotic mixture (1%, Sigma) and new born calf serum (NCS) (10%, Sigma Aldrich) at 37°C and 5% CO_2 as described before (Beckmann and Grevelding, 2010; Beckmann et al., 2012). For each experiment, 5–10 *S. mansoni* couples were kept per well in 6-well or 12-well plates with 2–3 ml culture medium per well. The medium was changed every 24 h. All following experiments were done in duplicate. To investigate the influence of serum albumin (SA), adult *S. mansoni* couples were maintained in culture medium, which contained 1.93 g/L SA due to the supplemented NCS. Because the *in vivo* concentration of albumin in blood of mice or humans is much higher with 44 g/L (mice) or 35–55 g/L (human), the culture medium was supplemented with BSA or HSA in a concentration of 43 g/L to achieve a final albumin concentration of 45 g/L, reflecting the *in vivo* situation. *S. mansoni* couples were treated in these different media (without additional SA, with BSA, or with HSA) with Imatinib (0, 10, or 50 μM) for 6 days.