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Rapid Communication

Imatinib has a fatal impact on morphology, pairing stability and survival of adult *Schistosoma mansoni* in vitro

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ABSTRACT

Schistosomes cause bilharzia (schistosomiasis), one of the most prevalent parasitic diseases for human and animals worldwide. Praziquantel (PZQ) is the only widely used drug for treatment and control of this parasitemia. Since a vaccine is not yet available, and in light of emerging resistance against PZQ, the search for alternatives has high priority. Here we present that Imatinib, a compound used in human cancer therapy (Gleevec; STI-571), significantly affected schistosome morphology and physiology in vitro. Besides its negative effect on gonad development and pairing stability, Imatinib led to pathological alterations of the gastrodermis, which finally caused the death of the parasite.

Protein-tyrosine kinases (PTKs) represent a large group of signal transduction molecules which are widely distributed in the animal kingdom, controlling diverse cellular processes such as adhesion, migration, proliferation and differentiation (Hubbard and Till, 2000). PTKs catalyse the transfer of the γ-phosphate of ATP to tyrosine residues of specific cellular proteins. This biochemical modification modulates enzymatic activity and creates binding sites for the recruitment of downstream signalling proteins. Two classes of PTKs are present in cells: transmembrane receptor PTKs (RTKs) and cytoplasmic PTKs (CTKs). Depending on their function and state of activation, CTKs are located in the cytoplasm, they are anchored to the inner leaflet of the plasma membrane as parts of protein complexes, or they occur in the nucleus (Takahashi et al., 2009). In vertebrates, CTKs were grouped in eight distinct families: Src, Syk, Jak, Abl, Fak, Fes/Fer, Csk and Btk (Hubbard and Till, 2000).

Due to their key role in cellular signalling pathways, the catalytic activity of CTKs is strictly regulated. However, alterations of the activity of CTKs induce physiological changes which can contribute to cancer development. Three mechanisms have been described by which CTKs become constitutively activated leading to cancer: over-expression, activating mutations or chromosomal translocations. One prominent example is the reciprocal translocation between chromosomes 22 and 9 [t(9;22)], which results in the formation of the Philadelphia chromosome (Ph-chromosome) in humans. This translocation causes the deregulation of the cAbl-kinase leading to primary events in the genesis of chronic myelogenous leukaemia (CML), and of other types of human leukaemias (Deininger et al., 2000).

In recent years PTKs have become the pharmaceutical industry's most studied class of drug targets, and a number of protein kinase inhibitors have been generated and approved for cancer treatment in humans (Cohen, 2009). Beyond this, PTKs have also gained interest as targets for anti-parasitic treatment strategies to fight *Plasmodium falciparum* (Ward et al., 2004), *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania* spp. (Naula et al., 2005) or *Schistosoma mansoni* (Dissous et al., 2007; Knobloch et al., 2007). In *S. mansoni* CTKs of the Src- (Kapp et al., 2004), Fyn- (Kapp et al., 2001), Syk- (Knobloch et al., 2002) and Fes-families (Bahia et al., 2007) have been cloned and characterised. They are expressed in larval and/or adult stages. Expression in adults was localised in a variety of tissues such as the subtegument (SmTK5, in part SmTK4), the parenchyma (SmTK5, in part SmTK3 and SmTK4), the gastrodermis (SmTK5) or the reproductive organs of both genders (SmTK5, SmTK3 and SmTK4, the latter only in ovary and testes). Recent studies with Src- or Syk-specific CTK inhibitors indicated pivotal roles for these kinases in gonad development. Treatment of adult schistosomes with the Src-kinase specific inhibitor Herbimycin A led to a significant reduction of mitotic activity and egg production in the female, and morphological aberrations in the vitellarium (Knobloch et al., 2006, 2007; Beckmann et al., 2010a). The Syk-kinase inhibitor Piceatannol caused dramatic defects in oogenesis and spermatogenesis of adult schistosomes (Beckmann et al., 2010b).

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By yeast two-hybrid analysis we recently identified the new CTK SmTK6 of *S. mansoni* (GenBank Accession No. FN397679) as an upstream binding partner of SmTK4 (Beckmann et al., 2010b). According to database analyses SmTK6 showed homology to Src, but also to Abl kinases. To elucidate whether SmTK6 may represent the only Abl-like kinase in schistosomes we searched through the *S. mansoni* genome data set (www.sanger.ac.uk; Berriman et al., 2009) and identified partial sequences of Abl kinases within three contigs (Smp_169230, Smp_169240, Smp_128790). The first two sequences (Smp_169230, Smp_169240) are located next to each other on one scaffold (Smp_scaff000392) and encode one Abl kinase (SmAbl1). The third contig (Smp_128790; scaffold Smp_scaff000020) encodes a second Abl kinase (SmAbl2).

Using total RNA of adult worms, the full-length cDNA sequences of *SmAbl1* (4,992 bp; GenBank Accession No. FN582310) and *SmAbl2* (3,927 bp; GenBank Accession No. FN582311) were amplified in overlapping fragments by reverse transcription PCR (RT-PCR) (data not shown), cloned and sequenced. The deduced amino acid (aa) sequences (SmAbl1 1663 aa, SmAbl2 1308 aa) contain predicted SH3 (Src homology 3) domains (SmAbl1 aa 49–160, SmAbl2 aa 56–118), SH2 domains (SmAbl1 aa 239–322, SmAbl2 aa 165–248), and tyrosine kinase domains (SmAbl1 aa 428–681, SmAbl2 aa 283–535), respectively. Multi-alignment and phylogenetic analyses (Supplementary Fig. S1) revealed that both molecules belong to the class of Abl kinases. At the aa sequence level the identity of SmAbl1 and SmAbl2 is only 24%. Within the catalytical tyrosine kinase domain, however, the identity of both kinases is 67% (Supplementary Fig. S1A). In situ-hybridization analyses using probes against non-conserved regions demonstrated transcriptional activity of SmAbl1 and SmAbl2 in both genders (Fig. 1). Under high stringency washing conditions (0.1× SSC), *SmAbl1* and *SmAbl2* transcripts were observed close to the area of the ootype, presumably including cells of the vitelloduct and oviduct (Fig. 1C; results not shown). Under medium stringency conditions (0.5× SSC) transcripts were also detected in the ovary (Fig. 1A, B, E and F), vitellarium (Fig. 1D and G), testes (Fig. 1A and E) and weakly in some cells of the parenchyma (Fig. 1A–G) as well as the gastrodermis (Fig. 1D and G). From these results we deduced that Abl kinases may play a role in reproduction, but may have other physiological functions. To analyse whether known Abl-kinase inhibitors may affect adult *S. mansoni*, we tested Imatinib (Imatinib mesylate, $C_{29}H_{31}N_7O{\cdot}CH_3SO_3H$), an Abl-kinase-specific inhibitor, which is also known as Gleevec (or STI-571) and used in human cancer therapy (Manley et al., 2002; Larson et al., 2008).

Imatinib is a small-molecule inhibitor acting as a competitive antagonist of the ATP binding site of Abl. The drug blocks the binding of ATP and phosphorylation of substrates. This leads to the functional inactivation of the kinase and interrupts downstream signalling processes. Besides Abl kinases, Imatinib also blocks the functional activity of the transmembrane receptor cKIT and platelet-derived growth factor receptors (Manley et al., 2002). However, searching through the *S. mansoni* genome data set (Berriman et al., 2009) we found no evidence for the existence of homologues of both molecules. Therefore, we expected that Imatinib has a specific effect on SmAbl1/2 and/or SmTK6. It appears likely that the schistosome Abl (Abl-like) kinase could be inhibited by Imatinib, since conserved aa residues that were shown in the human Abl kinase to interact with Imatinib (Nagar et al., 2002) are also present within the tyrosine kinase domain of SmAbl1/2 and SmTK6. From the 21 residues, 20 were perfectly conserved in SmAbl2, whereas 18 and 15 were conserved in SmAbl1 and SmTK6, respectively (Supplementary Fig. S1A).

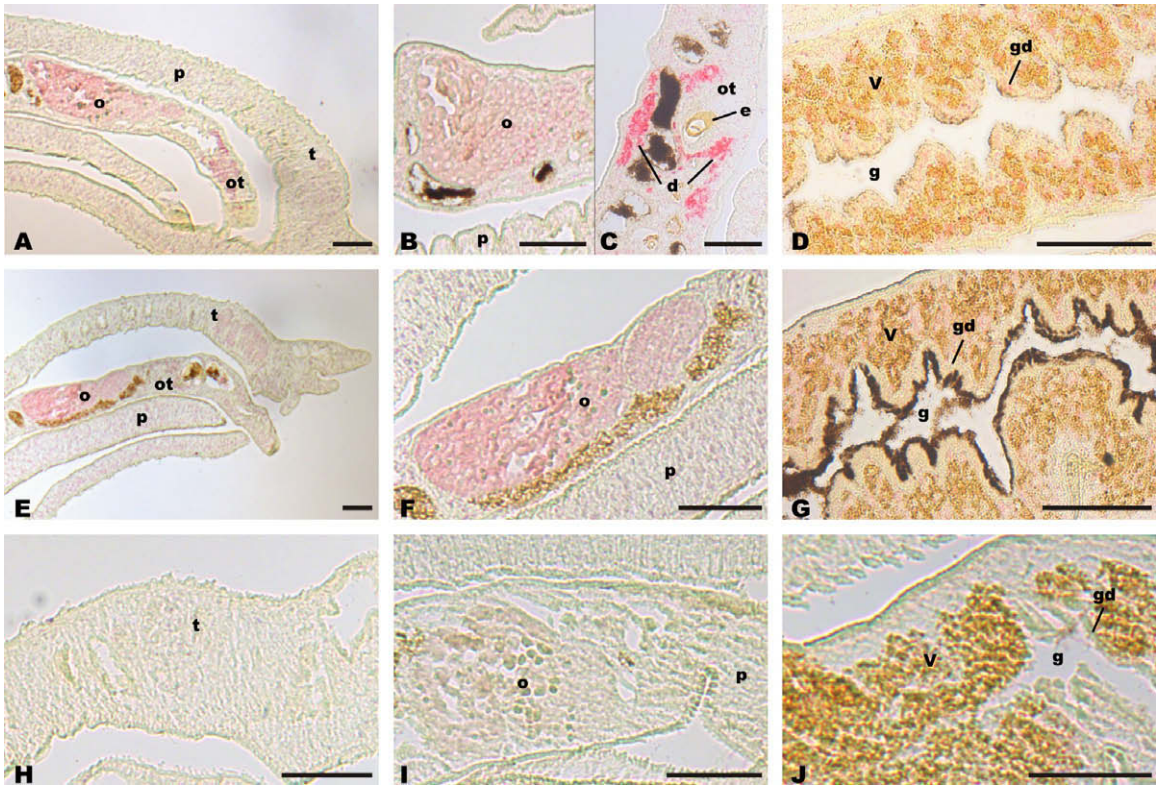

Fig. 1. In situ hybridizations of *SmAbl1* and *SmAbl2* in adult *Schistosoma mansoni*. Subclones of non-conserved regions of both Abl kinases (*SmAbl1*, A–D, nucleotide (nt) position 316–685 or 2119–2509; *SmAbl2*, E–G, nt position 1037–1355 or 1037–1435) served as templates to synthesize digoxigenin (DIG)-labelled anti-sense transcripts used for hybridization. Clear signals were detected in the area of the ootype (ot) including the ducts (d; vitelloduct, oviduct), and the ovary (o) of the female, as well as in the testes (t) of the male. Weak signals were observed in the vitellarium (v), and the gastrodermis (gd), as well as in some cells of the parenchyma (p) of both genders. For control, a DIG-labelled sense probe of SmAbl2 was used (H–J). e, egg; g, gut; scale bar, 50 μm.