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Letter to the Editor

Schistosomicidal effect of the anti-inflammatory drug diclofenac and its structural correlation with praziquantel

Sir,

Schistosomiasis, an important parasitic disease caused by blood flukes of the genus *Schistosoma*, affects more than 200 million people worldwide. Treatment and control of schistosomiasis is currently dependent upon a single drug, praziquantel, but after three decades of use in monotherapy, evidence of emerging drug resistance and low efficacy of praziquantel has been reported and for this reason new antischistosomal agents are urgently required [1,2].

Diclofenac is a well-known non-steroidal anti-inflammatory drug and it can significantly reduce the size of liver granulomas surrounding *Schistosoma mansoni* eggs formed by the host. Moreover, a recent study reported that diclofenac could be used successfully as a preventive agent against *S. mansoni* infection [3]. Here we describe for the first time the in vitro effect of diclofenac against juvenile and adult *S. mansoni* worms, using praziquantel as a benchmark. In addition, owing to their different therapeutic classes, diclofenac was compared structurally with praziquantel using shape and charge modelling and overlay optimisation, assuming that similar small molecules can produce similar biological activities given that they may bind similarly to the same biological receptor.

Schistosoma mansoni adult worms (BH strain) were maintained in *Biomphalaria glabrata* snails and *Mesocricetus auratus* hamsters. Host infection for parasite recovery, schistosome preparations and culture were performed as previously described [2,4]. Briefly, mice exposed to 400 cercariae were sacrificed at 21 days after infection for juvenile recovery, whilst mice exposed to 120 cercariae were sacrificed at 49 days after infection for adult recovery. Adult worm pairs or juveniles were incubated in a 24-well culture plate (TPP, St. Louis, MO) containing RPMI 1640 medium (Vitrocell, Campinas, SP, Brazil) supplemented with 10% foetal bovine serum (Vitrocell) at 37 °C in a 5% CO_2 atmosphere.

The drugs tested were diclofenac sodium (Fagron, São Paulo, Brazil) and praziquantel (Merck, São Paulo, Brazil). The drugs were dissolved in dimethyl sulphoxide (DMSO) and the survival of juvenile- and adult-stage worms was assessed in vitro by incubation with different concentrations of diclofenac. Negative controls using medium with 0.5% DMSO, and positive control media containing various concentrations of praziquantel were similarly evaluated [4].

The geometries of diclofenac and praziquantel were obtained through crystallographic structures deposited in the Crystallographic Cambridge Data Center. The molecules were computationally parameterised with atom types from the General AMBER Force Field (GAFF). Atomic charges were computed using the AM1 method, as implemented in the Antechamber program, plus desolvation. Molecular shape was described as a Gaussian function, with atomic parameters taken from GAFF. The overlay optimiser was the augmented Lagrangian method (local, no-derivative), and it was equal to 1 for the radius of Van der Waals (VdW) and 1 for electrostatic potential simultaneously. The similarity rate was calculated using the magnitude of the correlation coefficient squared times one hundred, employing the MolShaCS package [5].

The results show that diclofenac presents significant antischistosomal activities both for juvenile and adult stages of the parasite (Fig. 1A). Diclofenac was able to kill juvenile- and adult-stage schistosomes at concentrations of $\geq$3.25 and $\geq$6.5 μg/mL, respectively. Surprisingly, in relation to the juvenile stage, praziquantel was less potent than diclofenac.

Although praziquantel has been used to treat schistosomiasis for over 30 years, the detailed mechanism of its action remains unknown. Voltage-gated Ca^{2+} channels in the membrane are believed to be one of the possible targets for praziquantel. In this study, it was observed that diclofenac has a schistosomicidal effect, but the mechanism by which it does so has yet to be deciphered.

Taken together, considering that praziquantel and diclofenac are different molecules and that their schistosomicidal activities are different, it is reasonable to conclude that praziquantel and diclofenac reveal different modes of action. To investigate the differences between praziquantel and diclofenac, a structural analysis was performed to calculate the area of solvent accessible surface (SAS), the molecular electrostatic potential (MEP) map and the polar surface area (PSA), using the VEGA package (Supplementary Table S1). Despite significant differences in surface area, the most important difference between diclofenac and praziquantel concerns the polar surface, which is proportionally greater in diclofenac, whereas their hydrophobic surfaces are proportionately equal. The global similarity between diclofenac and praziquantel was calculated and showed ca. 70% similarity. The similarity between these molecules was also computed taking into account only the VdW radii and only electrostatic potential and the results were 75% and 32%, respectively. The overlay between praziquantel and diclofenac is shown in Fig. 1B.

Supplementary Table S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijantimicag.2014.06.018.

In conclusion, we have demonstrated that the non-steroidal anti-inflammatory drug diclofenac possesses in vitro antischistosomal activity against juvenile and adult *S. mansoni* worms. Furthermore, we can conclude that the similarity between the two molecules points to different hits despite the similar effects. This study opens up new avenues for the treatment and control of schistosomiasis using diclofenac, providing support for a previous study that found promising in vivo activity with diclofenac [3].

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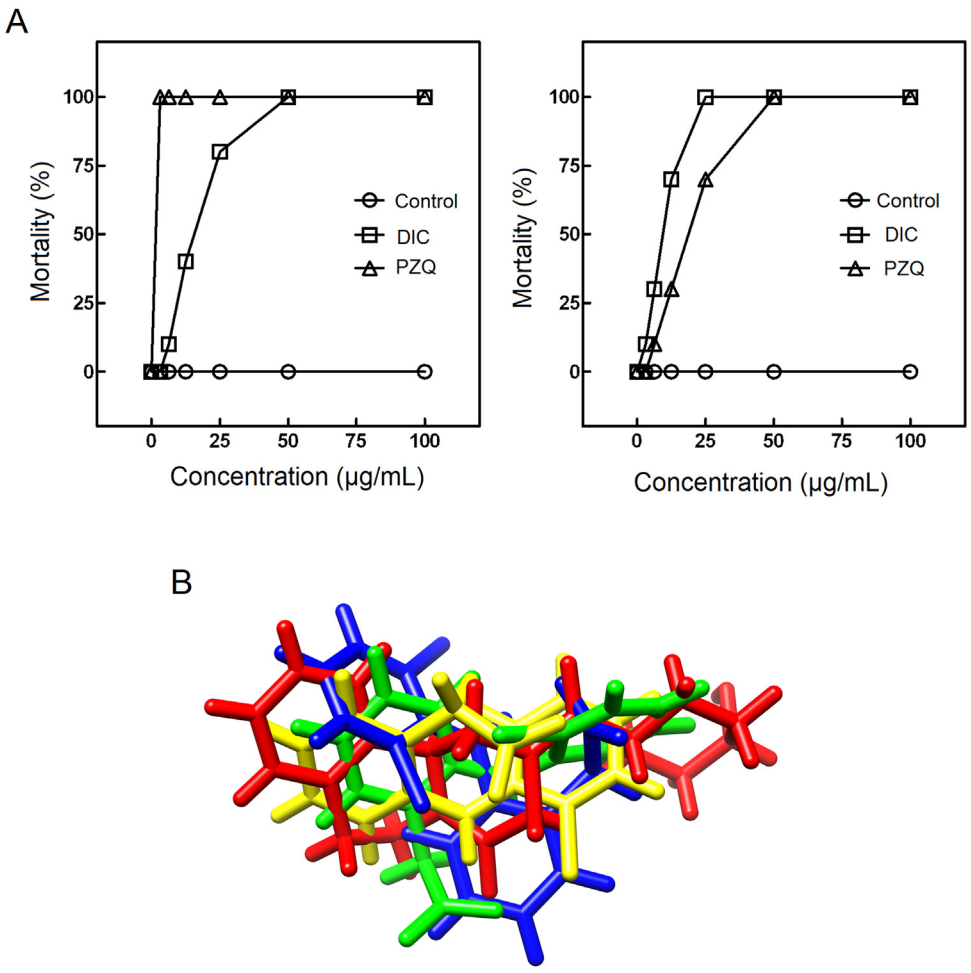

Fig. 1. Schistosomicidal effect of the anti-inflammatory drug diclofenac and its structural correlation with praziquantel. (A) Effects of diclofenac (DIC) and praziquantel (PZQ) at various concentrations on the mortality of adult (left) and juvenile (right) stages of *Schistosoma mansoni* following 24-h exposure in vitro. Results are expressed as the percent mortality in groups of 30–40 worms exposed to drugs. (B) Overlay between praziquantel and diclofenac. In red, praziquantel. In blue, diclofenac with correlation equal to 1 for the Van der Waals radius. In green, diclofenac with correlation equal to 1 for the electrostatic potential. In yellow, diclofenac with correlation equal to 1 for both variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

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