Schinella, Guillermo; Tournier, Horacio; Zaidenberg, Anibal
In vitro and in vivo activity of berberine on the blood trypomastigote from Trypanosoma cruzi
Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, vol. 6, núm. 3, mayo, 2007, pp. 81-85
Universidad de Santiago de Chile
Santiago, Chile

Available in: http://www.redalyc.org/articulo.oa?id=85660307
**In vitro and in vivo activity of berberine on the blood trypomastigote from Trypanosoma cruzi**

[Actividad de la berberina en tripomastigotes sanguíneos de Trypanosoma cruzi]

Guillermo SCHINELLA 1,2,*, Horacio TOURNIER 1,2, Anibal ZAIDENBERG 3

1 Cátedra de Farmacología Básica, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Calles 60 y 120, 1900 - La Plata, Argentina.
2 Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina.
3 IDIP – Instituto de Desarrollo e Investigaciones Pediátricas (Hospital de Niños ‘Sor María Ludovica’), La Plata, Argentina.

*Contact: schinell@uv.es

Received 20 May 2007. Accepted 30 July 2007

**Abstract**

Blood transfusion has been found to be an important mechanism in the transmission of the Chagas disease and there is a need for new agents able to inhibit the infectiveness of *Trypanosoma cruzi* in stored blood. The methanolic extract of the root of *Coptis chinensis* and the alkaloid berberine has been previously identified as effective trypanocidal agents both *in vitro* and *in vivo* and could be promising prophylactic drugs to address this issue. The extract of *C. chinensis* and its main bioactive, berberine, resulted more active against Bra C15C2 clone epimastigotes in axenic cultures than allopurinol, the antitrypanosomal drug of reference, with IC50 values of 1.7 µg/ml and 0.3 µg/ml (0.81 µM), respectively. The *in vitro* anti-trypanosomal activities of the methanolic extract of roots of *C. chinensis* and its main bioactive, berberine, were tested using epimastigotes of the clon Bra C15C2. Berberine (250 µg/ml) and crystal violet were equally effective in preventing transfusion-mediated infection of CF1 mice with the clon H510C8C3. However berberine (30 mg/kg/day, p.o.) for 30 days was unable to enhance the survival of already infected animals. The mechanism of protection may encompass both diminished parasitaemia in stored blood as well as inactivation of the infectiveness of the parasites, opening a new perspective in the fight against Trypanosomiasis.

**Keywords:** Trypanosoma cruzi; Coptis chinensis, berberine, blood transfusion, prophylaxis

**Resumen**

Las transfusiones sanguíneas son un importante factor de transmisión de la enfermedad de Chagas y se necesitan agentes profilácticos para inhibir la infección por *Trypanosoma cruzi* presente en los bancos de sangre procedentes de donantes infectados. *Coptis chinensis* y el alcaloide berberina han sido previamente identificados como efectivos tripanocidas tanto *in vitro* como *in vivo* y por tanto son potencialmente interesantes como agentes profilácticos. El extracto metanólico de raíces de *C. chinensis* y su principal compuesto activo, berberina, resultaron activos frente a epimastigotes del clon Bra C15C2 en cultivos axénicos, con IC50s de 1.7 µg/ml y 0.3 µg/ml (0.81 µM), respectivamente. Berberina (250 µg/ml) y violeta de genciana fueron igualmente efectivos en la prevención de la infección por tripomastigotes del clon H510C8C3 mediada por transfusión sanguínea en un modelo con ratones CF1. Sin embargo, berberina (30 mg/kg/día, p.o.) durante 30 días no pudo mejorar la supervivencia de ratones CF1 previamente infectados por transfusión. El mecanismo de protección podría implicar tanto una cierta citotoxicidad contra el parásito sin llegar a eliminarlo completamente en sangre almacenada; así como una inactivación del potencial infectivo del parásito, lo cual abre una nueva perspectiva en la lucha contra la Tripanosomiasis

**Palabras clave:** Trypanosoma cruzi; Coptis chinensis, berberina, transfusión sanguínea, profilaxis
INTRODUCTION

American trypanosomiasis or Chagas’ disease is after malaria, the most prevalent vector-borne illness in Latin America and is caused by a protozoan parasite, *Trypanosoma cruzi*, which is transmitted to humans by triatomin bugs. The disease is associated with poverty in rural areas in Central and South America. In 1996 it was estimated that between 16 and 18 million people were infected, of whom over 6 million would develop clinically overt disease and 45000 would die per year. (WHO, 2007). This microorganism has a complex life cycle with different developmental stages in different hosts. It multiplies inside mammalian cells as amastigotes and after that are released into the bloodstream as trypomastigotes; which can infect other cells or be ingested by the insect vector. In the gut of Triatomine, trypomastigotes differentiate to the other reproductive forms, epimastigotes; which at the rectal ampoule transform into the infective metacyclic trypomastigotes; which are unloaded with the bug’s excreta and to reach the bloodstream of the vertebrate hosts (Tyler and Engman, 2001). Blood transfusion is the second most important mechanism of transmission of Chagas’ disease (Docampo et al., 1988). This fact is of epidemiological importance and it has become a major health problem in South and Central America because of the migration of infected individuals into and out of the Americas (Schmunis et al., 2001).

Current chemotherapy recommended for the treatment and the prevention of Chagas disease has serious limitations because of their limited effectiveness and important drug-related side effects (Stoppani, 1999; Paulino et al., 2005). The exposed reasons make the search of new chemopreventive or chemotherapeutic agents an urgent priority.

One of the current approaches in the quest for new trypanocidal drugs relies on screening the biological activity of natural products. Different products with a very broad range of structural types have been assessed against *T. cruzi* in cultures or in infected animals (Sepúlveda-Boza and Cassels, 1996) and, among them, plant-derived products containing alkaloids have shown very promising anti-trypanosomal activity (Cavin et al., 1987; Rojas de Arias et al., 1994). We recently showed how the methanolic extract of roots of *Coptis chinensis* was able to inhibit the growth of epimastigotes of *T. cruzi* with a 25-fold higher potency than reference compounds normally used in these kind of screenings (Schinella et al., 2002). Berberine is one of the major protoberberine alkaloids present in extracts of *C. chinensis* and it has a good demonstrated anti-protozoal activity in different models (Phillipson and Wright, 1991; Cavin et al., 1987; Abe et al., 2002, 2004).

This research deals with the evaluation of the trypanocidal properties of berberine against epimastigotes in axenic cultures and blood trypomastigotes of different clones of *T. cruzi* as well as its potential as prophylactic agent in transfusion mediated trypanosomiasis.

MATERIALS AND METHODS

Chemicals

Culture mediums were from Gibco BRL (Life Technologies, NY, USA). Berberine and other chemicals for the assays were of analytical grade (Sigma Co., St. Louis). The plant material was purchased from Asia Natural Products (Amposta, Spain) and certified by The School of Medicine of the University of Beijing as fitting the pharmaceutical standards for its use in Traditional Chinese Medicine (macroscopic characters, microscopic characters and berberine content of 5-7% according the Chinese Pharmacopoeia). The methanolic extract of the root of *Coptis chinensis* was prepared as previously described (Schinella et al., 2002).

Animals

Female CF1 mice (c.a. 30 g each) from the Biological Institute (La Plata, Argentina) were used. They were kept in standard environmental conditions and fed with rodent diet with tap water *ad libitum*.

Analytical high - performance liquid chromatography (HPLC) - diode array detector (DAD) analyses.

HPLC-DAD analysis was performed on a Merck-Hitachi system equipped with a Pump L-6200, L-7455 Diode Array Detector and Auto Sampler L-7200, injection valve (Reodyne), loop of 100 μl, precolumn Lichrospher® C18 (4 × 4 mm, 5 μm, Merck), and column Lichrospher® C18 (250 × 4 mm, 5 μm, Merck). The data were collected and processed with the software DAD-Manager (Merck-Hitachi). The analysis of the extract was carried out with the following mobile phase: A (H2O + trifluoroacetic acid 0.01%) and B (methanol + trifluoroacetic acid 0.01%); elution profile: isocratic 40% B.
**In vitro antiprotozoal assay**

*T. cruzi* epimastigotes (clone Bra C15C2) were cultured in F29 media supplemented with 10% (v/v) heat-inactivated fetal calf serum at 27 °C, with an inoculum of $5 \times 10^5$ cells per ml. Compounds were added at different concentrations and all assays were carried out in triplicate. Final dimethyl sulphoxide (DMSO) concentration was always less than 0.5%. After 72 h of contact with the samples, parasites were stained with Wrigth-Giemsa and counted in a Neubauer chamber. The activity of the compounds was assessed by comparison with the negative control (DMSO) and allopurinol was used as positive control (Zaidenberg et al., 1999).

**Transfusion assays with pre-treatment of the infected blood**

Blood from CF1 mice infected with the clone H510C8C3 of *T. cruzi* was collected on the fourteenth day, when parasitaemia peaks (Zaidenberg et al., 1999). The final concentration of $1 \times 10^7$ trypomastigotes/ml of blood was obtained by diluting the samples with blood from healthy CF1 mice. The assays were performed in Eppendorf tubes with 400 µl of blood in the presence of berberine (250 µg/ml), gentian violet (250 µg/ml) or vehicle (DMSO) (Mafezoli et al., 2000). The microcentrifugue tubes were incubated 24 h at 4° C and the presence of parasites monitored by microscopy. The blood was then inoculated in healthy CF1 mice ($n = 3$ per group). Survival was monitored daily during a period of 60 days.

**Transfusion assay with post-treatment of the recipients**

Male mice CF-1 (20-25 g, three groups/10 animal each) were infected with $1 \times 10^5$ trypomastigotes of *T. cruzi* (clone H510C8C3). After 24 h the groups were treated with berberine (30 mg/kg/day, p.o.), benznidazol (100 mg/kg/day), or vehicle (0.2 ml de sterile, bidistilled water/day) for 30 days. Parasitaemia and survival were monitored during 40 days after the infection (Zaidenberg et al., 1999).

**Statistical analysis**

Data were expressed as mean ± S.D. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnett's $t$-test for multiple comparisons. Differences were considered significant at $P \leq 0.05$. The inhibitory concentration 50% (IC$_{50}$) was calculated from the concentration/effect regression line. In each case, an appropriate range of 3 – 4 concentrations was used. Kaplan Meir method was used for the analysis of the survival curve.

**RESULTS AND DISCUSSION**

The inhibitory activities of berberine and *C. chinensis* extract on the in vitro growth of the *T. cruzi* epimastigote form are shown in Figure 2. The concentration of the alkaloid producing half-maximal inhibition (IC$_{50}$) was 0.3 µg/ml (0.81 µM), one order of magnitude lower than that observed with the extract of *C. chinensis* (IC$_{50} = 1.7$ µg/ml).

**Figure 1.** Chemical structure of berberine.

**Figure 2.** (A) HPLC Chromatogram of the methanolic extract of *C. chinensis*. (B) HPLC Chromatogram of berberina in the same elution conditions (C) Spectra of the pure berberine and of the main peak in the extract.
Table 1. Mortality of the mice inoculated with infected blood pre-treated with berberine or crystal violet during a period of 60 days.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (DMSO)</td>
<td>3/3</td>
</tr>
<tr>
<td>Berberine</td>
<td>0/3</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0/3</td>
</tr>
</tbody>
</table>

According to the Chinese standards, the content in berberine of *Rhizoma coptidis* should be close to 70 mg/g of drug (Ji *et al.*, 1999) and correspondingly the WHO monographs also states that the content in berberine of this drug must be between 5-7% (WHO, 1999). The University of Beijing certified that our material was abiding these standards and we confirmed by HPLC analysis that berberine was present in the extract as one of the major peaks (see Figure 2).

It was previously reported that berberine is active against epimastigote of *T. cruzi* (Cavin *et al.*, 1987; Abe *et al.*, 2002). However, in the conditions of our assay, berberine shows higher potency than that observed by Cavin *et al.* (1987). Moreover, it was reported that berberine is able to immobilize all the epimastigote forms of *T. cruzi* after 48 h incubation at 26 °C at a concentration 300 µg/ml (Abe *et al.*, 2002) whilst in our conditions we observe the complete absence of viable forms of the parasite in the culture medium at only 100 µg/ml. These discrepancies are probably attributable to the different sensitivities of each strain of the parasite and/or the influence of the different culture mediums used in the assays, highlighting how carefully the data reported in literature have to be compared.

Although axenic cultures of *T. cruzi* epimastigotes are a useful model to identify active compounds against the parasite, it does not take into account the different sensitivity of the other stages of *T. cruzi* present in the vertebrate hosts. Blood transfusion has been found to be an important mechanism in the transmission of the parasite. Thus, compounds able to prevent transfusion mediated infections could constitute an invaluable clinical tool in the fight against trypanosomiasis.

Crystal violet –also called gentian violet– is a recognised trypanocidal compound currently used in clinics to prevent blood borne infections during transfusion. No acute toxic side effects are reported even after administration of large amounts of gentian violet-treated blood (Docampo and Moreno, 1990). However, the long-term toxicity of this agent for blood recipients is still an open issue (Moraes-Souza and Bordin, 1996) as well as having the unpleasant effect of colouring the patient’s skin. In our experimental conditions, crystal violet produced the total lysis of the parasites after 24 hours of incubation but some parasites were still observed in blood treated with the alkaloid. Nevertheless, no parasitaemia was observed in the animals inoculated with the treated blood samples and there was no difference in the survival between both groups of animals. Control animals showed a high level parasitaemia and started to die at the 29th day (see Table 1). This suggests that the parasites observed in the blood treated with the alkaloid were rendered unable to infect the transfusion recipients.

**Figure 3.** Effects on the growth of *T. cruzi* epimastigotes in axenic cultures incubated in the presence of *C. chinensis* extract and berberine.

**Figure 4.** Survival of CF1 mice transfused with infected blood. Mice were treated daily with benznidazole and berberine.

After establishing that berberine had antiparasitic activity against epimastigotes and trypomastigotes *in vitro*, we investigated the effects of the alkaloid on already infected mice. In our model, berberine was unable to delay the death of previously infected animals (see Figure 3). The lack of efficacy in the treatment could be attributable to different factors.
including an insufficient dose of the alkaloid, but benzimidazol, at higher doses, did not protect the mice either. It has been reported that the bioavailability of berberine is quite poor in experimental models in dogs and rats and P-glycoprotein has been implicated (Pan et al., 2002) This alkaloid also has delay the emptying of the stomach in human volunteers (Xin et al., 2006). New experiments with higher doses are necessary in order to establish if berberine can provide an alternative to current drugs against the infection with T. cruzi.

CONCLUSIONS

To our knowledge this is the first communication of the activity of berberine as a prophylactic agent in transfusion experiments using the blood trypomastigote form of T. cruzi. Berberine failed to cure mice already infected by transfusions, but this alkaloid effectively protected transfusion recipients when used as a prophylactic drug in stored blood. Berberine somehow renders the parasites unable to proliferate in vivo by a not yet determined mechanism, thus opening new perspectives in the fight against Trypanosomiasis.

REFERENCES


