Evaluation of anti-Trypanosoma cruzi activities of nitrosyl/nitro ruthenium complexes in vitro and in vivo

Bastos T. M., Barbosa M. I. F., Meira C. S., Batista A. A., Soares M. B. P. CPqGM-Fiocruz, Salvador - Brazil, Universidade Federal de São Carlos, Departamento de Química, São Carlos - Brazil, CBTC – Hospital São Rafael, Salvador - Brazil.

Enznidazole has been used to treat Chagas disease since the decade of 70ies. Both drugs are effective in acute phase or recent chronic phase of the disease, but they can induce side effects for the patients. Therefore, more efficient drugs with lower toxicity are need for the treatment of this disease. Synthesis of transition metal compounds, especially ruthenium, has increased over the years and data have shown many biological applications of these compounds. The discovery of pharmacological functions of nitric oxide (NO), mainly on infection by protozoa, has led the development of NO donor compounds as therapeutic agents. This study aimed to evaluate the anti-T. cruzi activity of the nitrosyl/nitro ruthenium complexes, cis-[RuCl(NO$_2$)(dppb)(5-mebipy)], cis-[Ru(NO)$_2$][dppb](5-mebipy)], ct-[RuCl(NO)(dppb)(5-mebipy)](PF$_6$)$_2$ and cc-[RuCl(NO)(dppb)(5-mebipy)](PF$_6$)$_2$; (dppb=1,4-bis(diphenylphosphino)butane; 5-mebipy=5,5’-dimethyl-2,2’-bipyridine). The cytotoxicity assay was performed using splenocytes from BALB/c mice in the presence of the complexes at different concentrations. Trypanocidal effects were evaluated in vitro and in vivo. The evaluation of anti-T. cruzi activity in vitro was performed by incubating trypomastigotes and epimastigotes forms in the presence of the complexes. In vivo experiment was performed using female BALB/c mice infected with $10^4$ parasites per mouse and orally treated with 25 mmol/kg/day of each compound, daily, for 5 consecutive days. Scanning electron microscopy was performed to evaluate ultrastructural effects of the most active compound. The LC$_{50}$ values of cis-[RuCl(NO$_2$)(dppb)(5-mebipy)], cis-[Ru(NO)$_2$][dppb](5-mebipy)], ct-[RuCl(NO)(dppb)(5-mebipy)](PF$_6$)$_2$ and cc-[RuCl(NO)(dppb)(5-mebipy)](PF$_6$)$_2$ were 34.43 μM, 16.34 μM, 34.05 μM and 27.96 μM, and the IC$_{50}$ values for trypomastigotes were 8.38 μM, 2.87 μM, 2.08 μM and 5.85 μM, respectively. The first compound was not active against epimastigote form and the IC$_{50}$ values of the other complexes were 16.64 μM, 5.69 μM, 26.66 μM, respectively. IC$_{50}$ values of benznidazole, under the same conditions, were 11.41 μM and 10.68 μM. In vivo treatment with the compounds reduced parasitaemia and the groups treated with the compounds cis-[RuCl(NO$_2$)(dppb)(5-mebipy)] and ct-[RuCl(NO)(dppb)(5-mebipy)](PF$_6$)$_2$ increased the survival of BALB/c mice in acute phase of the Chagas disease. The complex ct-[RuCl(NO)(dppb)(5-mebipy)](PF$_6$)$_2$ presented the best results in vitro and in vivo. Scanning electron microscopy demonstrated that the treatment of trypomastigotes with this compound caused membrane fragmentation, surface discontinuities and shrinkage of the parasites. In conclusion, we observed that ruthenium complexes were effective against T. cruzi using in vitro and in vivo experimental models.

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