TRYPANOCIDAL ACTIVITY OF ORGANOTIN CHLORIDES ON *TRYPANOSOMA BRUCEI*-INFECTED MICE

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Summary :

The organotin compounds dibutyltin (DBTC) and diphenyltin dichlorides (DPTC) were tested for trypanocidal activity on a *Trypanosoma brucei*-infected mice model. At a dose of 10 mg DBTC and 15 mg DPTC/kg/day for five consecutive days, they cleared the parasites from the peripheral blood of the infected mice. Subinoculation of some healthy mice with the homogenates of liver, spleen, kidney, cerebrospinal fluid and blood from the mice considered cured, showed a few cases of relapse. The LD50 of DBTC and DPTC are 90 mg/kg and 75 mg/kg respectively.

KEY WORDS : organotin, *Trypanosoma brucei brucei*, trypanocidal.

Résumé : ACTIVITÉ TRYPANOCIDE DE L'ORGANOTINE CHEZ DES SOURIS INFECTÉES PAR *TRYPANOSOMA BRUCEI*

Les composés de l'organotine, dichlorides de dibutyltine (DBTC) et de diphenyltine (DPTC) ont été testés pour leur activité trypanocide sur un modèle de souris infecté par Trypanosoma brucei. *Aux doses de 10 mg de DBTC et 15 mg de DPTC/kg/jour cinq jours de suite, les parasites ont disparu du sang des souris infectées. Après sub-inoculation de souris en bonne santé avec des homogénats de foie, rate, rein, liquide cérébrospinal et sang de souris considérées comme guéries, quelques cas de rechute ont été observés. Les DL50 des DBTC et DPTC sont respectivement de 90 mg/kg et 75 mg/kg.*

MOTS CLÉS : *organotine,* Trypanosoma brucei brucei, *trypanocide.*

Organotins are compounds which possess one or more direct tin-carbon covalent bond(s) that are responsible for the specific properties of such molecules. The compounds assumed commercial significance in the 1970's. They are toxic to a variety of organisms including bacteria, fungi, protozoa etc. (Pain & Cooney, 1998). A large number of organotin compounds show reproducible antitumor activity in mice (Briddle & Gray, 1989). Of the four classes of organotin compounds, diorganotins, R_2SnX_2 are the largest group of tin studied for antitumor activity. Dibutyltin dichloride is reported to have *in vivo* effect against Ehrlich ascites tumor and IMC carcinoma, (Crowe, 1987; Crowe *et al.*, 1984). Diphenyltin dichloride-3,4,7,8-tetramethyl(-1,10-) phenanthroline is active against P.388 leukaemia and renal carcinoma (Atassi, 1985).

African trypanosomes are protozoan parasites that cause sleeping sickness in humans and related diseases in cattle. African trypanosomiasis is considered to be one of the most serious diseases affecting both man and his domestic animals and has been the bane against animal production in humid and subhumid tropical Africa.

Chemotherapy is still the main method for controlling the disease. So far there exists only one report on trypanocidal activity of an organotin compound (Nok *et al.*, 1992). Herein we report on the trypanocidal activity of other organotins– dibutyltin dichloride (DBTC) – $(C_4H_9)_2SnCl_2$ and diphenyltin dichloride (DPTC) – $(C_6H_5)_2SnCl_2$.

MATERIALS AND METHODS

Dibutyltin dichloride (DBTC) and diphenyltin dichloride (DPTC) were synthesized as described by Van der Kerk & Luijten (1956). The organotin compounds were dissolved in solutio petit (26:33:42) ethanol, glycerol and water respectively as described by (William *et al.*, 1977).

Six weeks old BALB/c mice were purchased from the Department of Pharmacology and Clinical Pharmacy Ahmadu Bello University, Zaria and the *T. b. brucei* (stabilate EATRO 410) was obtained from the Department of Veterinary Parasitology and Physiology, Ahmadu Bello University, Zaria, Nigeria.

The mice were intraperitoneally infected with 10^2 trypanosomes. Treatment started six days post-infection and members of group I and II were intraperitoneally treated with 10 mg DBTC/kg/day and 15 mg DPTC/kg/day respectively for five consecutive days until the parasi-

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taemia disappeared. The group III was treated with placebo (solutio petit) and served as control. The trypanocidal effect of DBTC was assessed by determining the level of parasitaemia every twenty four hours after treatment. Both dosages were selected from a preliminary work which represented the minimal dose that elicits trypanocidal effect.

Three mice each from the DBTC- and DPTC-treated groups were sacrificed three weeks post-treatment. Homogenates of their liver, spleen, kidney and cerebrospinal fluid (CSF) were used for subinoculation experiment on healthy mice. About 0.5 ml of blood was recovered from each of the treated mice and used to inoculate three healthy mice each. All tissues (liver, spleen and kidney) were removed under sterile conditions, washed, weighed and macerated separately in equal volumes of PBSG. Aliquots of 0.1 ml of each organ homogenate in PBSG was inoculated into healthy mice. The parasitaemia was then routinely checked for four weeks as described by Nok *et al.*, 1994.

RESULTS

The results are shown in Tables I and II.

Six days post-infection with *T. b. brucei*, the parasitemia in the experimental and control mice developed to 10^5/ml of blood. On the sixth day of infection the mice showed signs of early trypanosomal infection. On the commencement of treatment at 10 mg DBTC/kg and 1 mg DPTC/kg six days post-infection, there was an observed gradual decrease in the level of parasitaemia until the fifth day of treatment when no parasites were observed in the blood.

Table I shows the results of studies of relapse three weeks after treatment with 10 mg DBTC/kg and 15 mg DPTC/kg. 83.3 % of the treated mice showed no parasitaemia in each batch. The control group III showed high parasitaemia, and died as a result. Also, of the apparently cured mice, 23 % in group I and 42 % in group II were observed to relapse into the infection with low parasitaemia three weeks after treatment with DBTC and DPTC respectively. This observation, even with very low parasitaemia, suggests incomplete recovery, which could be due to incomplete treatment regimen. However, it shows the benefit of clearing the blood of the parasites as compared to the control group III.

Group	Studies of relapses in mice three weeks post-treatment with DBTC and DPTC		
	No of mice	Aparasitaemic	Cured
I	12	83 %	67 %
II	12	83 %	58 %
III	8	0 %	–

Group I: Mice infected and treated with DBTC 10 mg/kg × 5.
Group II: Mice infected and treated with DPTC 15 mg/kg × 5.
Group III: Infected control.

Table I – Activity of DBTC and DPTC against *T. brucei* infected mice.

Group	Number of parasitized mice				
	Liver	Kidney	Spleen	CSF	Blood
DBTC-treated	1	0	0	0	0
DPTC-treated	1	0	1	0	0

The number of DBTC- and DPTC-treated mice killed for subinoculation is three in each treated batch.

Table II. – Parasitized mice following the subinoculation into healthy mice of tissue homogenate from DBTC- and DPTC-treated mice.

Table II shows the development of parasitaemia following the subinoculation of healthy mice with tissue homogenates from the DBTC and DPTC-treated mice. It is observed from the table that no parasites were detected in the healthy mice subinoculated with the homogenates of kidney, cerebrospinal fluid (CSF) and blood of the DBTC and DPTC-treated mice. One healthy mouse from each of the three subinoculated with the liver homogenate of the DBTC and DPTC-treated mice showed very low parasitaemia four weeks after subinoculation. Also a mouse out of the three subinoculated with the homogenate of spleen from the DPTC-treated mouse showed parasitaemia four weeks after subinoculation.

DISCUSSION AND CONCLUSION

Following the administration of 10 mg DBTC/kg, six days post-infection for five consecutive days, trypanosome parasites were eliminated from the circulation. The organotin DPTC at a dose of 15 mg/kg for five days also cleared the blood of the parasites from the onset of the treatment. The logarithm plot of parasitaemia against time following treatment, follows a first order rate kinetics. Extrapolated half life ($t_{1/2}$) values show that the $t_{1/2}$ of the parasites in response to the effects of DBTC and DPTC are 0.80 and 0.83 day respectively.

The trypanocidal activity of bis(tributyltin oxide) had previously been reported (Nok *et al.*, 1992). Also it has been shown that the activity of organotin compounds depends on RxSn moiety (Crowe *et al.*, 1984; Barbieri *et al.*, 1982; Humer *et al.*, 1985). In a supportive experiment, subinoculation on the healthy mice with blood, CSF, and homogenates of liver, kidney and spleen of the apparently cured mice revealed residual parasites in the liver and the spleen during the aparasitaemic

period. The reappearance of the parasites could be due to incomplete treatment regimen. Histopathological analysis did not show any significant damage to the organs of the DBTC and DPTC-treated mice. Moreover the LD_{50} of both compounds is between 75 and 90 mg/kg (data not shown).

Developing an effective trypanocidal drug effective against all species of trypanosomes is a difficult task. Moreso, the same arsenical trypanocides have been used for the past 50 years and resistance against these drugs have recently registered growing frequency in Western and Eastern Africa (Boudichon, 1998). In an attempt to exploit the trypanocidal potentials of organotins, DBTC and DPTC were tested and found to be trypanocidal against the trypanosomes. DBTC and DPTC may be promising compounds in the treatment of human or animal trypanosomiasis. However, because of the incomplete clearance of the *T. b. brucei* from all tissues of the infected mice at the dosage used, a combination therapy with other trypanocidal compounds could enhance the efficacy. Synthesis of organotin compounds with different substituent groups other than chloride or more hydrophobic components could be of advantage.

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