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Trehalose - 6- phosphate-potent anti-onchocerciatic agent

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Summary

Onchocerciasis is a disease that engages the attention of most researchers. Presently, there is not an ideal onchocerciatic agent, hence the search must continues. Consequently α, α - trehalose - 6- phosphate was synthesised and assessed for onchocerciatic activity against *O. volvulus;* using diethylcarbamizine citrate as the control drug. Results from this study showed that α, α- trehalose-6-phosphate is a glucose analogue with effective micro and macro-filaricidal agent, better than that of the control drug. The inhibitory action of this compound on enzyme trehalase is â postulate for the mechanism of action of trehalose-6-phosphate. The structure activity relationship of this new compound is fully discussed. This study postulates that this compound could be used to eradicate onchocerciasis both in man and animals.

Keywords: *Trehalose, onchocerciasis, O. volvulus, Diethylcarbamizine*

Résumé

L'onchocercose est une malaide qui attire l'attention de la plupart des chercheurs, elle est globelement une maladie problematique. Presentement, il n'ya a pas d'agent ideal d'onchocercose deja identifie, par consequent il ya necessitè pour la recherche de continuer. L'α - α - trehalose –6-phosphate avait ètè synthetisè et evaluè pour son activite contre O. Volvolus, en utilisant le Diethyl Carbamazine Citrate comme medicament control. Les resultats de cet etude montrent que l' α - α trehalose –6-phosphate est un analogue du glucose ayant une response antimicro-onchocerciatique plus effective, et mmeilleur que celle du medicament controle. L'action inhibitrice de ce composé sur l'enzyme de la trehalose est postulé comme le mechanisem d'action de la structure et de l'activite de ce nouveau compore sons entriement discuté dans cet article. Cet étude suggere que ce composé peut etre utilisé pour le traitement de l'onchocercose chez les hommes et less animaux.

Introduction

Onchocerciasis is a disease that engages the attention of most researchers being globally problematic. Diethylcarbamizine citrate and ivermectinm, which are currently being used in the clinics to treat this disease are microfilaricidal agents, hence the search for an ideal onchocerciatic agent must continue. Biochemical studies of the filarial nematodes have shown that the major energy producing process in different stages of the life-cycle of filarial nematodes is glycolysis. More research in this area resulted in a postulate that inhibition of filarial glucose transport was a valid chemotherapeutic target for the treatment of onchocerciasis since there is a significant difference in the glucose transport system in filarial worms and that of the host [1]. Also in 1981, it was noticed that some glucose analogues [2] were able to inhibit the uptake of carbohydrates in worm. These facts prompted the development of a compound, a glucose analogue also able to inhibit glycolysis in the parasitic nematodes, but without adverse effects in the host. Consequently, α-α-trehalose-6-phosphate was synthesised and screened for the anti-onchocerciatic response in *O. Volvulus*.

Materials and methods

(i) Male microfilariae and adult *Onchocerca Volvulus* used in this study were obtained from Vom Veterinary Hospital Vom Jos, Nigeria and identified by the Superintendent of the same establishment.

(ii) The incubating medium for this study was 50.0mls of 20% inactivated calf medium (G11CO) while

(iii) α-α-trehalose-6-phosphate was obtained as follows:

Equimolecular quantities of α-α-trehalose dihydrate (A) and diphenyl chlorophosphate were heated together in a distillation flask in presence of dry pyridine to produce 6-diphenyl-trehalose-6-phosphate (B) which on further acetylation with glacial acetic acid gave 6-diphenyl α, α-trehalose-6-phosphate hepta acetate (C). On dehydrogenation of (C) with Adam's catalyst and further deacetylation in 1M NaOH solution, α-α-trehalose-6-phosphate (Scheme 1) was obtained. Spectroscopic analysis (NMR, and IR) was used to assign the correct molecular structure to this new compound as shown in (a) and (b).

(a) ***Equipment***

(i) Varian XL-300 spectormeter for infra-red determination (IR);

(ii) Bruker WP805Y spectrometer operated at 20.14 MH_2C for NMR analysis.

IR spectra (KBr disc)cm 2920 - 2830 = CH; 1670-1630 C = 0; γ_{max} cm 1030-1040 P = 0 - alkyl.

(b) H-NMR ($CDCL_3$)

δ(7.99)m = CH_2; δ(4 9) = OH-alcoholic; δ(2.2) = OH; δ(4.4) = CH_2; δ(1.4) = C-P)

(c) pka of this new compound was determined by the potentiometric method (Oke 1986) and found to be pka_1 = 10.32; pkb_2 = 8.11. M.pt. = 198° $[\alpha]_D$ = +197°

(2) Evaluation of Microfilaricidal response of α, α-

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trehalose-6-phosphate on *O. Volvulus*

The procedures were as follows: sets of wells were labelled A, B, C, D, & E, each set containing four wells. Set A, wells were filled only with incubating medium, while Set B wells contained 500 μM/ml of α, α-trehalose-6-phosphate in incubating medium. Diethylcarbamaizine was the control drug in this study. Set C wells contained incubating medium inoculated with a solution of 500.o μM/ml of α, α-trehalose-6-phosphate dissolved in Tween 81 incubating and which had been previously seeded with 138 male micro filariae of *O. volvulus*. Set D wells contained 50 ml of Tween 81, incubating medium and 138 male microfilarial worms. Set E wells contained the following: 500 μM/ml diethylcarbamazine citrate dissolved in Tween 81 and 138 male micro filariae of *O. volvulus* inoculated into the incubating medium. This dose regimen was administered daily for 3 days. All sets of wells were incubated at 37°C under an atmosphere of 5% CO_2 in air, during the period of study. At the end of 72 hours, contents of wells, A,B,C, and D were observed [4] under the high-powered electron microscope to determine the larvicidal activity of the tested compounds. The results obtained were analysed by student "t" [5] analysis of variance [6] and recorded in Table 1.

(3) Evaluation of Filaricidal Activity of α, α-Trehalose-6-phosphate on Macrofilariae of ***O. Volvulus***

The same procedures as stated in (1) above were repeated to determine the anti-onchocerciatic macrofilaricidal activity of α, α-trehalose-6-phosphate on macrofilariae of *O. volvulus* but with the following alterations: (a) In this investigation 154 male adult worms of *O. volvulus* were used. (b) New sets of wells and fresh incubating medium were employed in the study. A total of 500μM/ml of α, α-trehalose-6-phosphate dissolved in Tween 81 was inoculated in sets C wells which have been formerly seeded with 154 male macrofilariae of *O. volvulus* and administered once daily for 3 days. Similarly, Set E wells containing incubating medium into which had been seeded 154 male adult worms *O. volvulus* were latter inoculated with 500 μM/ml of diethylcarbamazine citrate dissolved in Tween 81. This dose regimen was similarly administered once daily for three days as in (1) above, All other procedures in (1) above were strictly followed. All result were analysed by students "t" test [5], analysis of variance [6] and displayed in Table 1.

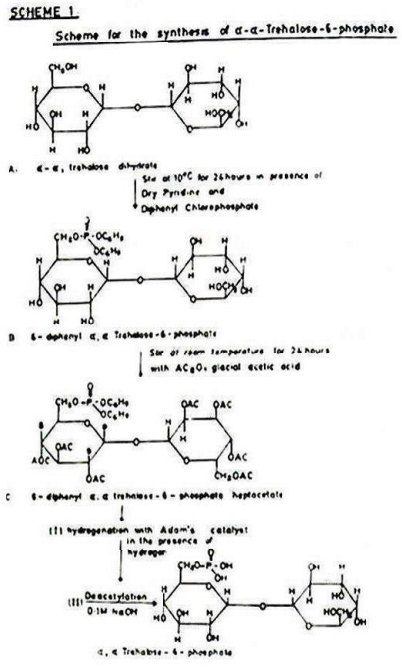

Fig. 2: Suppression produced by inoculating (T_6P) α, α- Trehalose - 6 - phosphate into a medium containing worms of O volvulus

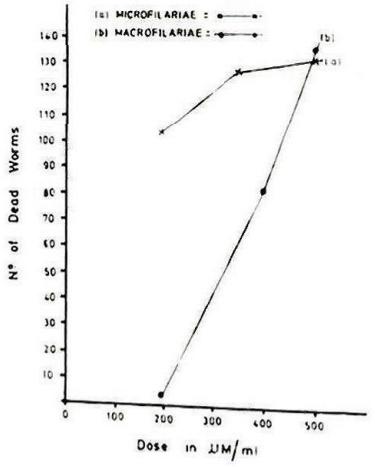

Table 1: Comparative filaricidal response and percentage chemotherapeutic efficancy of *O. volvulus* to diethylcarbamiazine citrate and trehalose-6-phosphate after 72 hours of treatment

		Viable microfilairal count (± S.D.)				Viable macrofilarial count (± S.D.)			
Compound	Dose	Before treatment	After treatment	D	Percentage chemotherapeutic Efficancy	Before treatment	After treatment	DD	Percentage chemotherapeutic Efficancy
D.E.C.C.	200 M	138.0 ± 4.0	118 ± 4.0	20	14.49%	154 ± 4.0	154.0 ± 5.0	0	0.0%
T6P		138.0 ± 3.0	35 ± 4.5	103	74.63%	154.0 ± 4.0	150.0 ± 5.0	4	2.5%
D.E.C.C.	400 M	138.0 ± 4.0	100.0 ± 4.5	38	27.53	154.0 ± 3.5	150.0 ± 5.0	4	2.5%
T6P		138.0 ± 4.0	12 ± 4.0	126	91.30%	154.0 ± 2.0	73.0 ± 4.0	81	52.54%
D.E.C.C.	500 M	138.0 ± 5.0	80.0 ± 4.0	50	42.02%	154.0 ± 4.0	140.0 ± 5.0	14	9.090%
T6P		138.0 ± 4.0	8.0 ± 4.0	130	94.20%	154.0 ± 4.0	20 ± 5.0	134	87.10%

(1) Values of variation in the number of the viable worms (Micor and macrofilariae) are means S T.D of the number of rats in each group ($P < 0.05$)

(2) Student's "t"-test in diethylcarbamiazine is compared with trehalose-6-phosphate within the same dose-regiment and across different dose-regimen ($P < 0.001$)

(3) Percentage chemotherapeutic efficancy of the tested compounds on these worms are obtained when compared with the initial number of worms before treatment.

Key to abbreviations
D E C C - Diethylcarbamiazine
T6P - α,α-trehalose-6-phosphate.

Earlier in this study, preliminary test-dose of α, α-trehalose-6-phosphate was carried out. As a result of this study the maximum dose for the investigation at 500 μM/ml of α, α-trehalose-6-phosphate and diethylcarbamiazine citrate respectively. The results of this preliminary investigation is also included in Table 1.

Results

The results of this study are shown in Table 1 and Figure I (a & b), while scheme 1 shows the synthetic steps to obtaining α, α-trehalose-6-phosphate. The anti-onchocerciatic response of α, α-trehalose-6-phosphate was dose-dependent (Table 1), having the maximum activity when the medium was inoculated with 500 μM/ml of α, α-trehalose-6-phosphate daily for three days. At this dose-regimen after 72 hours, α, α-trehalose-6-phosphate was able to reduce the number of viable microfilariae and macrofilariae by 94.2% and 87.10% (from 138 to 8 microfilarae) and macrofilariae (from 154 to 30) respectively. Diethylcarbamizine citrate at the same dose-regimen exhibited 42% microfilaricidal and 9.0% macrofilaricidal chemotherapeutic efficacy. The Figure I (a&b) showed the comparative rate of suppression of the life cycle of the worms of *O. volvulus* by diethlycarbamizine citrate and the synthesised compound α, α-trehalose-6-phosphate. This is very significant ($P \leq 0.05$)

Discussion

At a dose-regimen of 500 μM/ml for three days, α, α-trehalose-6-phosphate attained a percentage chemotherapeutic efficacy of 94.2% as a microfilaricidal agent, and 87.5% chemotherapeutic efficacy as a macrofilaricidal agent. Obviously, α, α-trehalose-6-phosphate has established, by this high filaricidal response, its superiority over diethlycarbamizine citrate as an anti-onchocerciatic agent. (Table 1).

In 1958, it was reported [8] that most parasitic nematodes contained trehalose. In *O. volvulus*, trehalose [-1-α(-D-glucopyranosyl)-α-D-glucopyranoside] has been indicated in cattle onchocerciasis as an important short term energy-store and a kinetic intermediate in the glucose uptake process. Like its parent compound, trehalose, α, α-trehalose-6-phosphate has the "pseudo disaccharidic core" -D-glucopyranosyl- a characteristic unit of the pseudo-oligosaccharidic α-glucosidase inhibitors. On hydrolysis of α, α-trehalose-6-phosphate in 0.5 M HCl at 100 °C, -D-glucose was the major product obtained showing that only one glucose molecule was attached to the "core". Schmidt [8] in 1980 observed that the smaller the number of glucose units attached to the "core", the more pronounced was the inhibitory effect of the pseudo-oligosaccharidic α-glucosidase inhibitors on oligosaccharides such as sucrase and maltase. α, α-trehalose-6-phosphate possesses all the structural merits of a typical pseudo-oligosaccharidic α-glucosidase inhibitor, hence it will be reasonable to attribute the ability of α, α-trehalose-6-phosphate to reduce the number of viable worms at different stages of development of the target-organism to its inhibitory activity as a pseudo-oligosaccharidic α-glycosides inhibitor. Recntly, [9] it was reported that α, α-trehalose-6-phosphate possesses the ability to reduce blood glucose level in experimental animals, and that this compound is competitive inhibitor of trehalase Trehalase an enzyme found in many nematodes is responsible for the hydrolysis of trehalose – (a glucose analogue also found in nematodes) into smaller glucose unit which are later resorbed at the renal tubules of these worms [10]. This was collaborated by Eliander's observation in 1965 that yeast trehalase was inhibited by D-glucose and α,α-trehalose-6-phosphate [11] and that a high concentration of this compound is a competitive inhibitor of trehalose. Consequently, as observed ealier by Hayes [5] the synthesised compound is able to inhibit glycolysis at every stage of the life-cycles of the target organism, resulting in the death of majority of the worms.

Furthermore, Allen (1971) and Schmidt (1972) independently [12,2] reported that glucose analogues of acarbose were able to inhibit the uptake of glucose in parasitic worms. Trehalose-6-phosphate is chemically a glucose analogue. Consequently, the mode of action of this synthesised compound may not be unconnected with its ability to inhibit glucose uptake.

In conclusion, α, α-trehalose-6-phosphate, being a pseudo-oligosaccharic α-glycoside and a glucose analogue, able to inhibit glycolysis in the worms of *O volvulus*. This is the basis of the anti-onchocerciasis activity of this synthesised compound. Hence, α, α-trehalose-6-phosphate is a very potent anti-onchocerciasis agent, more potent than diethlycarbamizine citrate the control drug used in this work.

Work is in progress in our laboratory to bring this compound to the clinics to treact onchocerciasis in man and animals.

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