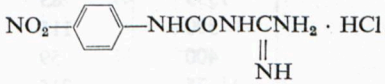
BIOLOGICAL ACTIVITY OF NITROGUANIL

T. URBAŃSKI[1], B. SERAFIN[2], D. F. CLYDE[3], K. JAKIMOWSKA[4], M. WUTKIEWICZ[4], P. NANTKA-NAMIRSKI[5], J. VENULET[4,6], G. O. SCHLÜTZ[6], J. SPŁAWIŃSKI and T. POTACZEK[7]

Abstract—Nitroguanil, the nitro-derivative of phenyl-amidineurea, was found to be an antimalarial of moderate activity and low toxicity in clinical trials. Furthermore, it was found to possess a potent anthelmintic activity in men. Nitroguanil exhibits also a molluscicide activity. Some pharmacological data of this compound are reported.

IN THE course of our investigations on new biologically active derivatives of amidine urea,[8] 1-(*p*-nitrophenyl)-2-amidineurea (I, T 72, Nitroguanil)[9] was found to have some interesting properties. It shows antituberculous activity *in vitro* but no favourable results in experimental tuberculosis were obtained.[10]



I

Structural similarity with Proguanil (Paludrine) led us to examine antimalarial properties of Nitroguanil. It is active *in vivo* against *P. gallinaceum* in doses about 3·5 times the dosis of Proguanil, and has 1/26-th of the toxicity of the latter.[11,12]

[1] Institute of Organic Synthesis, Polish Academy of Sciences, Warszawa, Poland.
[2] Institute of Technology, Warszawa, Poland.
[3] Malaria Service, Morogoro, Tanganyika; permanent address: London School of Hygiene and Tropical Medicine, London, England.
[4] Institute of Drugs, Warszawa, Poland.
[5] Pharmaceutical Institute, Warszawa, Poland.
[6] University of Damascus, Damascus, Syria.
[7] Sanatorium "Bystre", Zakopane, Poland.

[8] B. Skowrońska-Serafin and T. Urbański, *Tetrahedron* **10**, 12 (1960).
[9] T. Urbański, B. Skowrońska-Serafin and H. Dąbrowska, *Roczniki Chem.* **27**, 167 (1953).
[10] T. Urbański, B. Serafinowa, S. Malinowski, S. Ślopek, J. Kamieńska, J. Venulet and K. Jakimowska, *Gruźlica* **20**, 157, 293 (1952).
[11] Y. Ch. Chin, Y. Y. Wu, B. Skowrońska-Serafin, T. Urbański and J. Venulet, *Nature, Lond.* **186**, 170 (1960).
[12] Y. Ch. Chin, Y. Y. Wu, B. Skowrońska-Serafin, T. Urbański, J. Venulet and K. Jakimowska, *Bull. Acad. Polon. Sci., Ser. Sci. Chim.* **8**, 109 (1960).

Nitroguanil was also tested against *Trypanosoma gambiensae* in mice with good results.[13]

Antimalarial activity (clinical observations)

In view of low toxicity of Nitroguanil, steps were taken to test it against human malaria infections. Trials were carried out by one of us (D.F.C.) in Tanganyika in the area of hyperendemicity near Morogoro. The patients were semi-immune African children, 7–10 years old, infected mostly with *P. falciparum* (97·5%) but also with *P. malariae* (14·6%) and *P. vivax* and *P. ovale* (4·0%). The drug was administered orally; the results were compared with those obtained for cases treated with Proguanil and Chloroquine. The smallest dose of Nitroguanil sufficient to clear asexual parasitaemia within a period of 7 days was found to be 1333 mg (Table 1).

TABLE 1. ANTIMALARIAL ACTIVITY IN MEN (A)

Substance	Dose, mg	Cases treated	Cases cleared
Nitroguanil	935	85	77
	1066	75	69
	1200	91	89
	1333	83	83
Proguanil	300	115	113
	400	59	59
Chloroquine	75	215	212
	120	78	78

The mean clearance time for trophozoites of *P. falciparum* and the duration of protection (time reckoned from successful treatment to reappearance of trophozoites) were also determined (Table 2).

Neither signs of toxicity nor untoward effects were noted during the administration of Nitroguanil. Thus, a very large margin of safety was confirmed.

TABLE 2. ANTIMALARIAL ACTIVITY IN MEN (B)

Substance	Effective dose, mg	Mean clearance time, hr	Duration of protection, days
Nitroguanil	1333	76	10
Proguanil	400	64	9
Chloroquine	120	38	19

Anthelmintic activity in men

Anthelmintic activity of some antimalarial drugs as well as the fact that Nitroguanil acts as an inhibitor of folic reductase (according to observation of

[13] W. Jungstand, T. Urbański and B. Serafinowa, *Monatsberichte der DAW* **5**, 92 (1962).

one of us (P.N.-N.) prompted us to examine the substance in man against the following species of human worms:

1. *Taenia solium*
2. *Taenia saginata*
3. *Ascaris lumbricoides*
4. *Enterobus vermicularis*
5. *Trichocephalus dispar.*

Nitroguanil was administered orally (tablets 0·3 g) in two doses at a 1-hr interval, the total dose being 0·6–1·8 g. No purgation was required before or after the treatment, the elimination of worms with feces followed mostly on the same or, in few cases, on the next day.

Control of the fecal excreta was continued for 4 months after the administration. The results are collected in Table 3.

TABLE 3. ANTHELMINTIC ACTIVITY OF NITROGUANIL IN MEN

Parasite	Cases treated			Total dose, g	Cases cured
	Children		Adults		
	6–10 years	10–16 years			
Taenia saginata			5	1·2–1·8	5
Taenia solium			3	1·8	3
Ascaris lumbric.	10			0·6	10
		12		1·2	12
Enterobus vermic.	20			0·6	20
		11		1·2	11
			6	1·2	6
Trichocephalus dispar.		3		1.2	3
			2	1·8	2

Two groups of patients infected with *Enterobus vermicularis* showed reinfection 4 months after the successful treatment; in the third group which was protected against reinfection, the positive effect of the cure outlasted the observation period. In two cases of infection with *Taenia solium*, the first treatment failed, but the cure occurred with the second administration of Nitroguanil repeated after 3 months.

Except some mild forms of nausea, vomiting, and headache (11 cases, 15%) no important side effects were observed during the administration of Nitroguanil.

Thus, the successful treatment of all kinds of worm infections investigated in our experiments showed that Nitroguanil can be considered as a new promising anthelmintic. In further trials, the optimum dosage, mode and form of administration, will be established.

More particular data will be published separately.[14]

[14] J. Spławiński, *Pol. Tyg. Lek.* (in press).

Molluscicide activity

As a result of the experiments carried out with Nitroguanil it was shown that the LD_{50} concentration for different kinds of snails is *ca.* 20 p.p.m. which indicates a definite activity. This property seems to be rather specific because fish survive about 48 hr in concentrations as high as 80 p.p.m.

Chemically, the substance shows some relation to the new and powerful molluscicide, 5,2′-dichloro-4′-nitrosalicylanilide; nevertheless, it does not possess the salicylanilide structure claimed by Gönnert *et al.*[15] as indispensable for this specific activity. Because of its use in medicine for other purpose the substance can be considered nontoxic; therefore, water containing this substance in molluscicidal concentrations is safe to drink.[16]

Pharmacology

The following pharmacologic tests on Nitroguanil were carried out:

(1) Subacute toxicity and pathological examination of internal organs of animals (stomach, liver, spleen and kidney) 6 weeks after administration of Nitroguanil.

(2) Action upon the arterial pressure and respiration.

(3) Action upon smooth muscles of isolated rabbit intestines according to Magnus.

(4) Determination of the level of Nitroguanil *in vitro* and *in vivo*.

(5) Influence on cell respiration of the rabbit's minced liver.

Ad (1) Subacute toxicity was examined in rats in doses of 200 and 100 mg/kg during 6 weeks.

The dose of 200 mg/kg daily was lethal for all the animals after 32 days of administration. The pathological findings were: maximum distended stomach overfilled with the contents, empty intestines and their walls congested.

The dose of 100 mg/kg daily during 6 weeks was also toxic. After 6 weeks, the amount of red cells was reduced to 50%; no pathological changes were found to occur in the amount of white cells, hemoglobine level and in urine.

Ad (2) The dose of 2·5 and 5·0 mg/kg of Nitroguanil did not exert any important influence on blood pressure and respiration; higher doses (7·5 and 15·0 mg/kg) lowered blood pressure.

Ad (3) Nitroguanil showed a definite spasmolytic action in the concentration of 0·02 mg/ml; the solution of 0·2 mg/ml solved the spasm induced by 0·2 mg/ml of ACh.

Ad (4) The method* of determination involves reduction of the nitro group present in Nitroguanil with zinc dust in hydrochloric acid solution followed by

[15] R. Gönnert and E. Schraufstetter, *Trop. Med. and Malaria* **2**, 197 (1959).

[16] J. Venulet and G. O. Schlütz, *Experientia* (in press).

* Z. Margasiński (private comunication).

treatment with sodium nitrite and coupling of the diazo product with N-(1-naphthyl)-ethylenediamine. The developing violet colour is evaluated in a Pulfrich colorimeter. The results *in vitro* are given in Table 4.

TABLE 4. SERUM NITROGUANIL LEVEL *in vitro*

Dose	Concentration of Nitroguanil, μg/2 ml					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
100 μg in 4 ml of blood	43·1	49·3	44·4	49·0	44·6	45·2

The experiment *in vivo* showed a significant difference as compared with the calculated concentration and the values found experimentally 3 hr after the administration of the drug (Table 5).

TABLE 5. SERUM NITROGUANIL LEVEL *in vivo* (A)

Dose p. o., mg/kg	Concentration in μg/2 ml	
	calc.	found
25	*ca.* 300	7·0 (2·3%)
50	*ca.* 600	21·0 (3·5%)
100	*ca.* 1200	32·0 (2·7%)

The level of Nitroguanil *in vivo* 6 hr after administration was also investigated (Table 6).

TABLE 6. SERUM NITROGUANIL LEVEL *in vivo* (B)

Dose p.o., mg/kg	Concentration in μg/2 ml					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
25	5·5	7·0	5·5	2.0	2·0	2·0
50	12·6	21·3	20·7	20·2	16·3	10·1
100	19·5	29·1	32·3	29·8	26·6	16·6

Thus, the level of the drug in the blood serum rises stepwise to a maximum reached between the 2nd and 3rd hour after administration. The difference between the drug level calculated and found seems to be caused either by the rapid secretion of the substance or by the detoxication process, or by both.

Ad (5) Influence on cell respiration of the minced liver of the rabbit is shown in Table 7.

TABLE 7.

Oxygen consumption in μl/100 mg tissue;
Nitroguanil dose 100 μg/0·2 ml /100 mg tissue

Control	Propylene glycol	Nitroguanil
$\bar{x}_1 = 72{\cdot}0$	$\bar{x}_2 = 47{\cdot}6$	$\bar{x}_3 = 57{\cdot}1$

By using the t-Student test it is possible to state that Nitroguanil does not exert any essential influence on the oxygen consumption of the liver cells.

Details of pharmacological investigations will be published separately.[17]

[17] K. Jakimowska, M. Wutkiewicz and J. Venulet, *Acta Physiol. Pol.* (in press).