Structure and Activity of Thiazole-Type Schistosomicidal Agents

I. NABIH▲, F. EL-HAWARY, and H. ZOOROB

Abstract □ The schistosomicidal agent 1-(5′-nitro-2′-thiazolyl)-2-imidazolidinone caused a rise in the α-keto acid level when given orally to rats. This effect could be diminished upon parallel administration of high doses of the vitamin thiamine. The biological effect of the drug could also be canceled through similar administration of the vitamin.

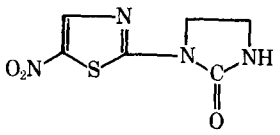
Keyphrases □ Schistosomicidal agents, thiazole type—1-(5′-nitro-2′-thiazolyl)-2-imidazolidinone, structure–activity, compared to thiamine, biological studies □ 1-(5′-Nitro-2′-thiazolyl)-2-imidazolidinone—structure–schistosomicidal activity, biological studies, compared to thiamine □ Thiazole-type schistosomicidal agents—1-(5′-nitro-2′-thiazolyl)-2-imidazolidinone, structure–activity, compared to thiamine, biological studies □ Thiamine—effect on oral administration of 1-(5′-nitro-2′-thiazolyl)-2-imidazolidinone, structure relationships □ Niridazole—structure–schistosomicidal activity, biological studies, compared to thiamine

In a previous communication, the schistosomicidal activity of a new agent, 2-*N*-(2′-methyl-4′-amino-5′-pyrimidoyl)amino-5-nitrothiazole was reported (1). The rational approach for investigation of its structure was based upon its close relation to thiamine; an attempt was made to profit from the concept of metabolic antagonism in this area of chemotherapy (1).

Recently, the compound 1-(5′-nitro-2′-thiazolyl)-2-imidazolidinone, niridazole (I), was found to possess potent schistosomicidal activity. The agent is structurally constituted of a thiazole moiety (a structural constituent of thiamine) attached to imidazolidinone which is involved in the chemical structure of the vitamin biotin (2).

It is well known that pyruvate metabolism can be influenced in a number of ways, one of which is vitamin B_1 deficiency. The latter, in the form of its pyrophosphate derivative cocarboxylase, has been proven to act as a coenzyme in pyruvate metabolism. Deficiency in vitamin B_1 may be due to dietary deficiency or, secondarily, to antagonism between thiamine and structurally related compounds. The latter may interfere with the enzyme system that converts thiamine to cocarboxylase or it may replace cocarboxylase in the pyruvate metabolism system (3, 4). Thus, the level of the blood pyruvate could be taken as an index for the visualization of such an action.

The aim of the present studies was to investigate the possibility of the occurrence of any antagonizing effect of the drug (I) for the biological activity of thiamine that would cause any rise in blood pyruvate.



I

BIOCHEMICAL STUDIES

Groups of five albino rats of the Sprague-Dawley strain were used. The weights of the rats varied from 170 to 230 g.; all rats were kept on a well-balanced stock diet.

Compound I was given alone to one group in doses of 75 mg./kg. of body weight (experimental therapeutic dose). Another group received the same dosage of the drug together with high doses (0.5 mg./kg. of body weight) of thiamine hydrochloride (I + thiamine). A third group received the same dose of the drug along with high doses (50 mg./kg. of body weight) of the vitamin *d*-biotin (I + biotin). A fourth group received the high dose of thiamine hydrochloride, and a fifth group received the high dose of *d*-biotin to determine the effect of high doses of these two vitamins.

High doses exceeding the normal requirements were adopted to ensure their biological availability to different tissues to counteract the effect of the administered drug.

Estimation of α-Keto Acids—This was carried out according to the method of Friedemann and Haugen (5). Blood samples (about 1 ml.) were withdrawn from anesthetized rats through heart puncture. This procedure was followed to avoid any exercise, because exercise would lead to misleading high results in the blood α-keto acid level.

Solutions—*Compound I Solution*—A 750-mg. amount of the compound was suspended in 15 ml. of distilled water. Each milliliter of this solution corresponds to 50 mg. of the drug[1].

Thiamine Hydrochloride Solution—Thiamine hydrochloride (5 mg.) was dissolved in 10 ml. of distilled water. Each milliliter of this solution corresponds to 0.5 mg. of thiamine hydrochloride.

Biotin Solution—One milligram of *d*-biotin was dissolved in 20 ml. of distilled water. Each milliliter of this solution contains 50 mcg. of the vitamin.

The drug, alone or with the supplemented vitamin, was given in aqueous suspension through the mouth by force feeding using an all-glass syringe fitted with an adequate, small, polyethylene tube. After administration of the dose, 1 ml. of distilled water was put in the syringe and allowed to pass to the stomach to ensure almost complete administration of the dose.

Administration of the drug, alone or supplemented with either of the two vitamins, was performed every 2nd day over a period of 2 weeks. At weekly intervals, blood samples were collected. When the drug was supplemented with the concerned vitamin, the calculated volumes of each were mixed together and both were administrated concurrently.

The mean calculated percentage of total blood keto acids (in terms of mg. pyruvic acid/100 ml. blood) in the experimental groups of animals are diagrammatically represented in Fig. 1. The mean values for total blood keto acids in each group before any treatment were taken as the basal control values. Apparently the normal level of total blood keto acids in control albino rats is approximately 1.4 mg. pyruvic acid/100 ml. blood.

BIOLOGICAL STUDIES

Mice weighing between 20 and 30 g. were infected with cercariae of *Schistosoma mansoni* from more than one infected snail to assure bisexual infection. After the developing period to adult worms

[1] These solutions were prepared at each time of experimentation.

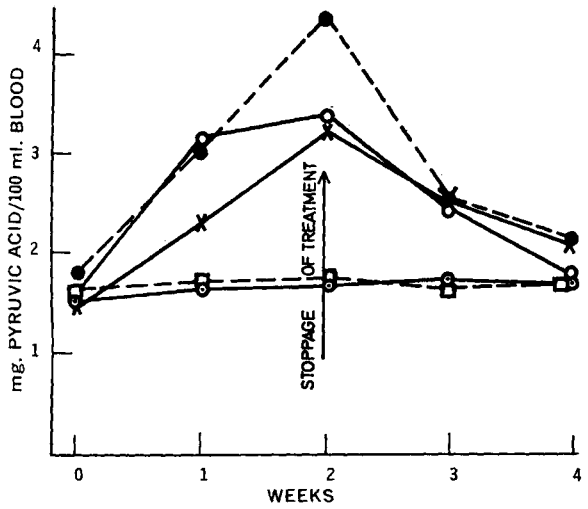


Figure 1—*Mean values for blood total keto acids (in terms of mg. pyruvic acid/100 ml.) in different groups of rats. The starting point in each curve indicates the basal control mean value for each group. Key:* O—O, *rats treated with I;* ●---●, *rats treated with biotin + I;* ×—×, *rats treated with thiamine + I;* □--□, *rats treated with biotin only; and* ⊙—⊙, *rats treated with thiamine only.*

(about 8 weeks), 24-hr. stools were collected and examined for ova. Those which showed positive living ova were isolated to be used in the experiments.

Thirty-six infected mice were divided into six groups:

Group a—Six mice were given the drug (I) alone in doses of 75 mg./kg. of body weight.

Group b—Six mice were given 75 mg. of the drug (I)/kg. of body weight, together with 0.5 mg. of thiamine hydrochloride/kg. of body weight.

Group c—Six mice were given 75 mg. of the drug (I)/kg. of body weight, along with 50 mcg. of *d*-biotin/kg. of body weight.

Group d—Six mice were given 0.5 mg. of thiamine hydrochloride/kg. of body weight.

Group e—Six mice were given 50 mcg. of *d*-biotin/kg. of body weight.

Group f—Six mice were kept as the control.

Administration of the drug alone or supplemented with either of the two vitamins, as well as administration of either of the vitamins, to the five groups was performed daily for 10 consecutive days.

Examinations for ova excretion in different groups showed that the drug (I) alone exerted its schistosomicidal effect in Group a, as evidenced by the absence of living ova in the collected stools.

In those infected animals in Group b, receiving thiamine along with the drug (I), clear inhibition of the schistosomicidal effect of the drug was observed and demonstrated by the continuation of living ova excretion in the stools of animals included in this group.

In the third group, which received the drug (I) along with biotin, the schistosomicidal effect of the drug still manifested itself as in Group a. Stool examination demonstrated complete absence of living ova.

In Group d, which received thiamine hydrochloride alone, a considerable rise in the ova count was observed during the observation period. Two weeks after the vitamin administration, the rise in the ova count returned to the control level existing before the administration of the vitamin.

In the fifth and sixth groups, which received biotin alone and the control, respectively, no change in the rate of ova excretion could be detected during the observation period.

DISCUSSION

Administration of I alone resulted in a considerable rise in the blood keto acid level (Fig. 1). This persisted on continuation of the drug administration. However, stopping the drug administration resulted in a drop in these levels; they returned to normal after 2 weeks.

Biochemical derangement in keto acid metabolism, as evidenced by the increase in the keto acid blood levels, was not observed in the group of rats given I + thiamine or thiamine alone. This indicates that the presence of thiamine in high amounts caused a decrease in the toxic effect of I in this connection.

Bueding and Fisher (6) reported that I activates phosphorylase phosphates of *S. mansoni*, causing an enhanced degradation of glycogen in the parasite, but no similar effect was demonstrated in the host (mice). Rapid degradation of the small amount of the parasite glycogen may partially share in causing the rise in the level of α-keto acids demonstrated in the blood. Possibly a competetive action between I and thiamine may exist due to the presence of the nitrated thiazole part in the drug molecule. Thus, administration of I alone would lead to blockage of the decarboxylation system. On the other hand, the concurrent administration of the high doses of thiamine seems to diminish such blocking action. This is supported by the marked diminution in the blood α-keto acid level when thiamine was given at high doses along with I.

However, when I + biotin was given, this toxic effect of I was evident, as was the elevation of the blood keto acid level. The marked rise in the levels of blood α-keto acids in animals receiving I + biotin is quite difficult to interpret. It had been reported (7, 8) that biotin deficiency reduced markedly the rate of oxidation of pyruvate. Furthermore, it had been demonstrated (9) that the addition of biotin to liver slices from biotin-deficient rats resulted in an increased utilization of pyruvate or lactate. This decreased utilization of pyruvate or lactate in biotin deficiency was reflected in a marked increase in the level of pyruvate in blood (10). The observed increase in blood pyruvate in rats receiving I + biotin and not in those animals receiving biotin alone could be accounted for by the antagonistic effect of I to the action of biological amounts of thiamine present in tissues, possibly because biotin may act as a synergistic factor to precipitate the toxic effect of the drug on the metabolism of α-keto acids.

From these findings, it appears that I causes an increase in the blood keto acids by antagonizing the biological function of thiamine and that concurrent administration of this vitamin with the drug diminishes the toxic effect of the latter. A similar biochemical disturbance may be initiated within the parasite and, hence, the schistosomicidal action of the drug is induced.

From the biological studies, it was determined that thiamine, when given in high doses along with the drug, diminished the schistosomicidal effect of the latter, whereas biotin did not exert such effect. Also, thiamine alone induced a considerable increase in the rate of ova excretion by the parasite.

These findings may add further support to the suggestions that I manifests its toxicity through interference with the biological function of thiamine and that the latter at higher doses could experimentally diminish such an effect.

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