# EFFECT OF PROLONGED ADMINISTRATION OF RIFAMPICIN AND ISONIAZID ON COMPONENTS OF GLYCOLYSIS AND ACTIVITY OF SOME ENZYMES OF CARBOHYDRATE METABOLISM IN THE BLOOD

A. G. Khomenko, I. V. Vysochina,
L. N. Efimova, and N. V. Kuz'mina

UDC 615.332+615.281:579.873.21].
015.42:[612.015.32+612.128

KEY WORDS: Antituberculosis drugs; carbohydrate and energy metabolism; enzyme activity; blood; erythrocytes.

The most soundly based theory [1] of the mechanism of the tuberculostatic action of isoniazid is the theory of lethal NAD synthesis, in which natural nicotinic acid is replaced by isonicotinic acid, and the structural analog of NAD thus formed cannot take part in NAD-dependent enzyme reactions. Isoniazid in the human body is acetylated by soluble liver N-acetyl transferase through acetylcoenzyme A with the formation of monoacetylisoniazid, which is further hydrolyzed to isonicotinic acid and acetylhydrazine [9, 10]. The latter, by means of the microsomal enzyme N-hydroxylating P450-dependent monooxygenase, forms free hydrazine, a competitive antagonist of pyridoxal phosphate and, consequently, of all pyridoxal-dependent enzyme reactions [2, 9, 10].

Rifampicin is a semisynthetic antibiotic which selectively inhibits the RNA-polymerase of *Mycobacterium tuberculosis*, which is similar to the RNA-polymerase of the mitochondria of higher animals. Rifampicin acts on synthesis of mitochondrial protein and, consequently, on mitochondrial function in man [3-5]. Rifampicin metabolism takes place *in vivo* in the liver microsomes with the formation of deacetylated and demethylated derivatives. It is excreted from the body mainly with the bile in the form of glucuronides, competing with bilirubin for UDP-glucuronyl transferase, which under clinical conditions may lead to hyperbilirubinemia [11-13].

A combination of rifampicin with other antituberculosis drugs and, in particular, with isoniazid increases the number of side reactions in clinical practice, especially those affecting the liver, and may lead to the development of jaundice, which is by nature both cholestatic and cytotoxic.

The object of this investigation was to study the prolonged action of therapeutic doses of isoniazid and rifampicin and their combination on some biochemical parameters in whole blood, plasma, and erythrocytes in rats.

## EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred male rats weighing about 250 g, receiving isoniazid (10 mg/kg), rifampicin (10 mg/kg), or both drugs together in the same doses, daily perorally for 6 months. Animals kept under identical conditions served as the control. The concentrations of lactate, pyruvate, ATP, ADP, and AMP, and also activity of lactate dehydrogenase (LDH) with its isozymes, malate dehydrogenase (MDH), and glucose-6-phosphate dehydrogenase (G6PDH) were determined in the blood after 6 months. The determinations were made by enzymic methods [15] (using test kits from Boehringer), in the writers' modification and by semimicro- and micromethods. To determine the chosen parameters in erythrocytes isolated from plasma and washed three times with 10 volumes of physiological saline, these techniques also were modified. The results were expressed in milligrams percent and in units per liter and subjected to statistical analysis [6, 7].

---

Central Research Institute of Tuberculosis, Ministry of Health of the USSR, Moscow.
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 4, pp. 54-57, April, 1982. Original article submitted July 27, 1981.

0007-4888/82/9304-0449$07.50  

TABLE 1. Concentrations of Lactate, Pyruvate, and Adenine Nucleotides (in mg%) in Blood of Control and Experimental Rats

| Parameter studied | Control (n = 14) | Isoniazid (n = 9) | Rifampicin (n = 8) | Isoniazid + rifampicin (n = 5) |
|---|---|---|---|---|
| Lactate | | | | |
| in blood | 29,82±3,25 | 24,6±3,68<br>$P>0,05$ | 33,9±5,40<br>$P\Phi=0,05$ | 29,10±1,92<br>$P>0,05$ |
| in erythrocytes | 12,46±2,29 | 13,3±2,31<br>$P>0,05$ | 18,08±5,51<br>$P_F<0,01$ | 8,83±2,18<br>$P>0,05$ |
| in plasma | 17,32 | 11,3 | 15,8 | 20,27 |
| Pyruvate: | | | | |
| in blood | 0,71±0,07 | 0,53±0,08<br>$P>0,05$ | 0,65±0,22<br>$P_F<0,01$ | 0,71±0,14<br>$P_F<0,05$ |
| in erythrocytes | 0,71±0,12 | 0,54±0,14<br>$P>0,05$ | 0,34±0,01<br>$P>0,05$ | 0,68±0,34<br>$P\Phi<0,05$ |
| Lactate/pyruvate (erythrocytes) | 17,55 | 24,63 | 53,2 | 13,0 |
| ATP (erythrocytes) | 26,03±2,66 | 22,89±2,83<br>$P>0,05$ | 25,28±3,31<br>$P>0,05$ | 20,84±3,21<br>$P>0,05$ |
| ADP (erythrocytes) | 1,82±0,18 | 2,67±0,64<br>$P_F<0,01$ | 2,33±0,78<br>$P_F<0,01$ | 2,41±0,78<br>$P_F<0,01$ |
| AMP (erythrocytes) | 0,16±0,007 | 0,44±0,03<br>$P_F<0,01$ | 0,35±0,09<br>$P<0,05$ | 0,28±0,13<br>$P_F<0,01$ |
| ATP/ADP | 14,3 | 8,6 | 10,8 | 8,6 |
| Total adenine nucleotides | 28,01 | 26,00 | 27,96 | 23,53 |
| Energy charge | 0,96 | 0,95 | 0,94 | 0,94 |

Legend. P) Significance by Student's test; PP) significance by Fisher's test.

## EXPERIMENTAL RESULTS

The lactate concentration was significantly increased in whole blood six months after daily administration of therapeutic doses of rifampicin, but when isoniazid was given there was a tendency for its level to fall, and combined administration of both drugs caused no change in the lactate concentration (Table 1). The lactate level in the plasma fell in animals receiving isoniazid by 35%, and in rats receiving rifampicin by 9%, but it increased by 19% following combined administration of the drugs. The changes were more marked in the erythrocytes: Under the influence of rifampicin the lactate concentration increased by 45%, but after combined administration of the drugs it fell by 30%. The pyruvate concentration in whole blood (mean values) was unchanged but Fisher's coefficient showed that the changes due to the action of rifampicin and a combination of the drugs were highly significant. The pyruvate level in the erythrocytes was significantly reduced by 48% by rifampicin, and pyruvate appeared in the plasma, which was not found either in the control or in the other experimental groups. The erythrocytic lactate/pyruvate ratio, which indirectly reflects the ratio between oxidized and reduced forms of NAD and NADH, was increased by 40% by isoniazid, threefold by rifampicin, but reduced by 26% by the combined action of both drugs.

The ATP content in the erythrocytes was not significantly changed but a tendency was noted for it to decrease in the rats receiving isoniazid and both drugs together (by 12 and 20% respectively). The content of ADP and AMP increased significantly in all animals, especially after administration of isoniazid (by 42% for ADP and threefold for AMP). The ATP/ADP ratio was reduced in all the experimental rats, the total adenine nucleotides decreased by 16% but only after combined administration of the drugs, and the energy charge of the system, after Atkinson [14], was almost unchanged. Enzyme activity in the blood also was changed by the action of both drugs, whether given separately or together (Figs. 1 and 2). Total LDH activity in the plasma was unchanged in absolute values, and only in rats receiving rifampicin was it reduced by 15%, although Fisher's test indicates "imbalance" of this system in all groups. The ratio between the hepatic and cardiac fractions of LDH in the plasma was changed: Under the influence of isoniazid activity of the hepatic form was increased, whereas under the influence of rifampicin activity of the cardiac form was increased. With a combination of the drugs, the action of isoniazid predominated in the plasma and activity of the "hepatic" fraction increased, with an increase in its relative percentage (Fig. 1). Rifampicin also increased total LDH activity in the erythrocytes, but also redistributed the fractions in the direction of an increase in activity of the cardiac form, and as a result the relative percentages changed from 58.9% (hepatic) and 42.9% (cardiac) in the control to 40.4 and 61.6% respectively (Fig. 2). Isoniazid increased the activity of both fractions (by 9 and 23%), but the hepatic isozyme remained predominant in the ratio between the fractions. A combination of

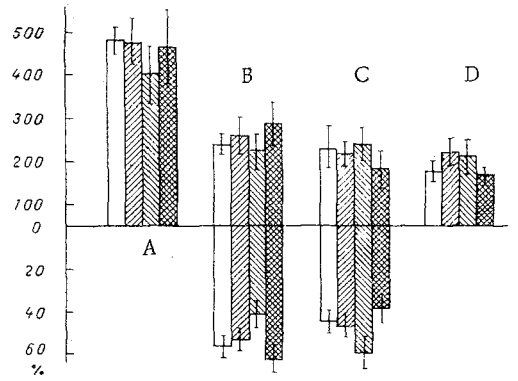


Fig. 1

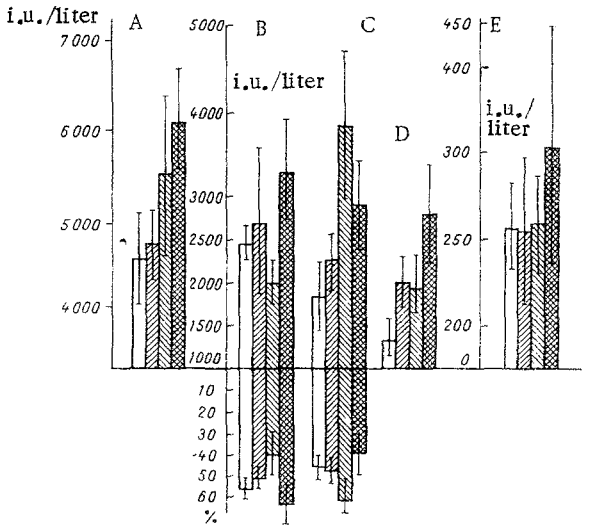


Fig. 2

Fig. 1. Enzyme activity in plasma: 1) control; 2) isoniazid; 3) rifampicin; 4) isoniazid + rifampicin. Ordinate: above — activity (in i.u./liter); below — relative percentages of LDH isozyme fractions. A) Total LDH activity; B) hepatic fraction; C) cardiac fraction; D) MDH.

Fig. 2. Enzyme activity in erythrocytes. Ordinate, A-D, 1-4) the same as in Fig. 1; E) G6PDH activity.

the drugs increased the activity of both fractions in the erythrocytes by 34 and 60% respectively, increasing still more the preponderance of the hepatic fraction. Total LDH activity in this group was increased by 34% (Fig. 2). Activity of the other NAD-dependent enzyme, MDH, in the plasma showed a tendency to increase when the drugs were given separately and to decrease when they were given together (Fig. 1). Its activity in the erythrocytes was increased in all experimental groups, especially during combined administration (twofold; Fig. 2). The function of the malate shunt in tissue cells is to transfer reducing equivalents from NADH from the mitochondria into the cytosol and vice versa. In erythrocytes, however, where there are no mitochondria and all energy metabolism is linked with glycolysis [15], the function of MDH is evidently rather different, although it is also coupled with NADH generation, for later utilization by glyceraldehyde phosphate dehydrogenase for the reversal of glycolytic oxidoreduction [8]. A fall in the ATP/ADP ratio in the erythrocytes causes activation of glycolysis, which requires an increase in the turnover of pyridine-nucleotide coenzymes NAD-NADH and, consequently, an increase in the activity of the enzymes connected with this turnover. Activity of G6PDH, the key enzyme of the pentose pathway of glucose oxidation, the primary function of which in erythrocytes is the generation of NADPH in order to maintain a stable level of reduced glutathione, which protects homoglobin against oxidation into methemoglobin, was significantly increased by 16% after combined administration of the drugs, although a tendency for its activity to change also was observed after administration of isoniazid alone. This increase in activity evidently points to an increase in the concentration of oxidizing agents in the erythrocytes and the necessity to protect hemoglobin against their action.

In conclusion, both drugs were found to activate NAD-dependent enzyme reactions, especially in erythrocytes. The fall in the ATP/ADP ratio in the erythrocytes is evidence of an increase in ATP turnover, and it evidently facilitates the intensification of glycolysis as the sole source of energy in these cells. In some cases the action of isoniazid and rifampicin is opposite, and when they are given in combination as a rule the effect of one of them predominates; its effect is often stronger than when given alone. The combined action of isoniazid and rifampicin in the erythrocytes gives rise to a state similar to hypoxia: an increase in the reducibility of pyridine nucleotides and a fall in the ATP/ADP ratio. However, the practically complete invariance of the energy charge (after Atkinson [14]) in the erythrocytes shows that their energy generation system is capable of performing its function.

LITERATURE CITED

1. G. A. Stepanskii and T. V. Selavri, in: Side Effects of Drugs (G. A. Stepanskii, ed.), [in Russian], Moscow (1969), p. 7.
2. É. S. Sodikov and K. S. Kazakov, Vopr. Med. Khim., No. 1, 3 (1978).
3. V. N. Adamovich, in: Antituberculosis Agents [in Russian], Moscow (1976), p. 23.
4. I. M. Bondarev and L. I. Zhigalina, in: Antituberculosis Agents [in Russian], Moscow (1976), p. 5.
5. V. I. Malyuk, V. I. Korzhov, and E. I. Suslov, Probl. Tuberk., No. 5, 62 (1978).
6. V. Yu. Urbakh, Biometric Methods [in Russian], Moscow (1964).
7. G. F. Lakin, Biometrics [in Russian], Moscow (1980).
8. M. N. Kondrashova and E. I. Maevskii, in: Regulation of Energy Metabolism and the Physiological State of the Organism [in Russian], Moscow (1978), p. 145.
9. M. R. Guchan and A. Horitz, Drug Metab. Rev., 1, 71 (1973).
10. P. Evans, in: Drug Metabolism: From Microbe to Man, London (1977), p. 346.
11. B. Inouye et al., J. Antibiot., 30, 494 (1977).
12. T. Wachi and B. Inouye, Kekkaku, 49, 239 (1974).
13. H. U. Bergmeyer (eidtor), Methoden der Enzymatischen Analyse, Weinheim (1974).
14. D. E. Atkinson, Biochemistry (Washi ton), 7, 4030 (1968).
15. S. Rappoport, Essays Biochem., 4, 69 (1968).

# ANALYSIS OF NEUROCHEMICAL MECHANISMS OF THE PSYCHOTROPIC ACTION OF TUFTSIN AND ITS ANALOGS

A. V. Val'dman, N. A. Bondarenko,
V. A. Kamysheva, M. M. Kozlovskaya,
V. N. Kalikhevich, and Z. A. Ardemasova

UDC 615.214.31.015.
4:612.822.1

KEY WORDS: tuftsin; tyrosine hydroxylase activity; psychotropic action.

Previous investigations [2, 3] have demonstrated the activating effect of tuftsin and some of its analogs on behavior and emotional reactivity, on the basis of which the psychotropic effect of these peptides has been linked with their action on the monoaminergic systems of the brain. The aim of the present investigation was to make a more detailed study of the catecholaminergic mechanisms of the central action of tuftsin (Thr-Lys-Pro-Arg) and its analogs Thr-Lys-Pro-D-Arg (D-Arg$^4$-tuftsin) and Leu-Lys-Pro-Arg (Leu$^1$-tuftsin), synthesized by V. N. Kalikhevich at the Chemical Research Institute, A. A. Zhdanov Leningrad University, with special reference to their effect on dopamine-dependent behavior and to tyrosine hydroxylase activity.

## EXPERIMENTAL METHOD

The action of the peptides on the dopamine systems was assessed on models of rotation behavior [14] in male Wistar rats weighing 200-250 g, with universal injury to dopamine terminals of the striatum (injection of 16 μg 6-hydroxydopamine — 6-OHDA — in 4 μl physiological saline, containing 0.2 mg/ml ascorbic acid, into the rostromedial part of the head of the right caudate nucleus 24 h previously). The number of rotations in 15 min was determined in a rotameter 35 cm in diameter. The character of stereotyped behavior [7] and the level of emotional reactivity [1] were estimated in points. Rats receiving an injection of 4 μl of the solvent served as the control. For statistical analysis of the data the nonparametric U criterion was used [4]. All drugs were injected intraperitoneally. Tyrosine hydroxylase (TH) activity was determined in structures of the corpus striatum and hypothalamus by a fluorometric method based on the rate of formation of the end product of the reaction — dopa. These results were subjected to statistical analysis by Student's t test.

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 4, pp. 57-60, April, 1982. Original article submitted November 18, 1981.

 0007-4888/82/9304-0452$07.50 