PLASMA XANTHINE OXIDASE AND RESISTANCE TO HYPOXIA: EFFECT OF PURINES AND ALCOHOL ADMINISTRATION

L. Novák, S. Štípek, T. Zima and S. Trojan*

*First Institute of Medical Chemistry and *Institute of Physiology*
Faculty of Medicine I, Charles University
Katerinská 32, 121 08 Prague 2
Czechoslovakia

SUMMARY

The administration of allopurinol significantly increased resistance to repeated hypobaric hypoxia, while hypoxanthine decreased it. The administration of adenine or inosine (25 mg/kg) was without effect. The results show pathogenic significance of xanthine oxidase-dependent production of free oxygen radicals in posthypoxic damage.

In other experiments, the administration of 50% ethanol (12 ml/kg) by gastric catheter increased plasma xanthine oxidase activity in both rats and hamsters.

KEY WORDS

plasma xanthine oxidase, hypobaric hypoxia, allopurinol, purines, ethanol

INTRODUCTION

A decrease in the Po_2 concentration in various tissues enhances the degradation of adenine nucleotides and increases the concentration of oxypurines (hypoxanthine and xanthine) in biological fluids /1/. Hypoxanthine and xanthine are substrates of xanthine oxidase (XOD; xanthine: oxygen oxidoreductase, EC 1.1.3.22), which oxidizes them to uric acid. Under pathological conditions (ischemia) this enzyme is probably converted from the D-form (xanthine: NAD^+ oxidoreductase) into its O-form, using dioxygen as electron acceptor /2/. During regeneration of the O-form of XOD in the presence of appropriate substrates (oxypurines), the superoxide and hydrogen peroxide are formed /3/. Such reduced oxygen forms may damage lipid and protein structures /4/.

During post-ischemic reoxygenation, a protective effect of allopurinol (4-hydroxypyrazol-3,4-d-pyrimidine) was observed /5/. Allopurinol is a competitive XOD inhibitor, used for treatment of clinical hyperuricemia. The extent of post-ischemic tissue damage was also diminished by administration of superoxide dismutase (SOD; EC 1.15.1.1) and scavengers of reactive forms of dioxygen /6/. This means that during post-ischemic reoxygenation, the tissue is damaged by the superoxide or by other species formed from it.

The aim of our work was to find out whether allopurinol and other purines would protect the organism not only from post-ischemic but also from post-hypoxic damage.

The functioning of the brain respiratory center has been proved to be the limiting factor of survival in hypoxia. During the final step before breath arrest the animal is unconscious. Mild hypoxia increases XOD substrates, such as hypoxanthine and xanthine, in body fluids /7/.

In some cases, XOD was reported to be a more sensitive marker of liver tissue damage than a variety of other enzymes /8/. Therefore, we also investigated the effect of ethanol on plasma XOD activity.

MATERIALS AND METHODS

Animals

Wistar rats of our own breeding stock, and hamsters, mice and guinea-pigs from the Velaz breeding farm, all females, 60-80 days old, were used.

Hypoxia

Hypoxia was induced in a hypobaric chamber. Rats were exposed to a simulated altitude of 7000 m (barometric pressure 41 kPa, Po_2 8.5 kPa) for 1 hour. A 25 min normoxic interval followed, at the beginning of which the animals were administered one of the following purines, intraperitoneally: hypoxanthine 25 mg/kg, adenine 25 mg/kg, allopurinol 50 mg/kg or inosine 25 mg/kg body weight. After normoxia, rats were exposed to a lethal hypoxic dose at a simulated altitute of 10,500 m (barometric pressure 24 kPa, Po_2 5.1 kPa) where the survival time was measured. The criterion for determining survival time was respiratory arrest. In experiments with four animals species, the pre-exposure was adjusted to 8000 m for 30 min, to get the shortest survival time measurable.

Hamsters proved to be more resistant to hypoxia than rats /9/; the measurable survival time was obtained by the following experimental procedure: pre-exposure at a simulated altitute of 8000 m (barometric pressure 36 kPa, Po_2 7.48 kPa) for one hour, lethal hypoxia at a simulated altitude of 11,000 m (barometric pressure 23 kPa, Po_2 4.2 kPa).

Some experiments were not preceded by hypoxia; in others, oxygen was applied during the interval between the two hypoxias at an overpressure of 5 kPa.

XOD measurement

XOD activity was measured by our own method adapted from the polarographic assay of oxypurines /10/. XOD activity was expressed in nmoles of oxygen consumed per second at 37°C (i.e., nkat), using the radiometer Po_2 electrode system.

Ethanol administration and measurement

50% ethanol was administered by gastric tube at the dosages of 8 ml/kg and 12 ml/kg body weight. Blood samples (puncture of myocardium by a special glass capillary) were obtained at 0, 90 and 120 min after administration. Ethanol was determined by gas chromatography accoding to Goldbaum /11/.

RESULTS

1. Effect of allopurinol, hypoxanthine, adenine, inosine and oxygen in rats exposed to interrupted hypoxia

Application of hypoxanthine after the initial hypoxic dose decreased the survival time of rats by an average of 20% in comparison with controls. Administration of allopurinol resulted in an average increase of 25%; no significant changes in survival time were observed after administration of adenine or inosine. Simultaneous administration of allopurinol and hypoxanthine or inosine increased the survival time more than allopurinol alone.

After the hyperoxic break, the survival time of controls was increased by a factor of about two. All the differences between allopurinol and other purines administration were more pronounced as compared to normoxia (Fig. 1).

In experiments without preliminary hypoxic exposure, neither allopurinol nor the other purines affected the survival time significantly (Fig. 2).

2. Effect of allopurinol, hypoxanthine, adenine, inosine and oxygen in hamsters exposed to interrupted hypoxia

Hypoxanthine application during the normoxic interval decreased the survival time, while application of allopurinol resulted in an increase (Fig. 3). All differences are significant, but less pronounced, as compared to rats. This is in agreement with different plasma XOD activities in rats and hamsters (Table 1).

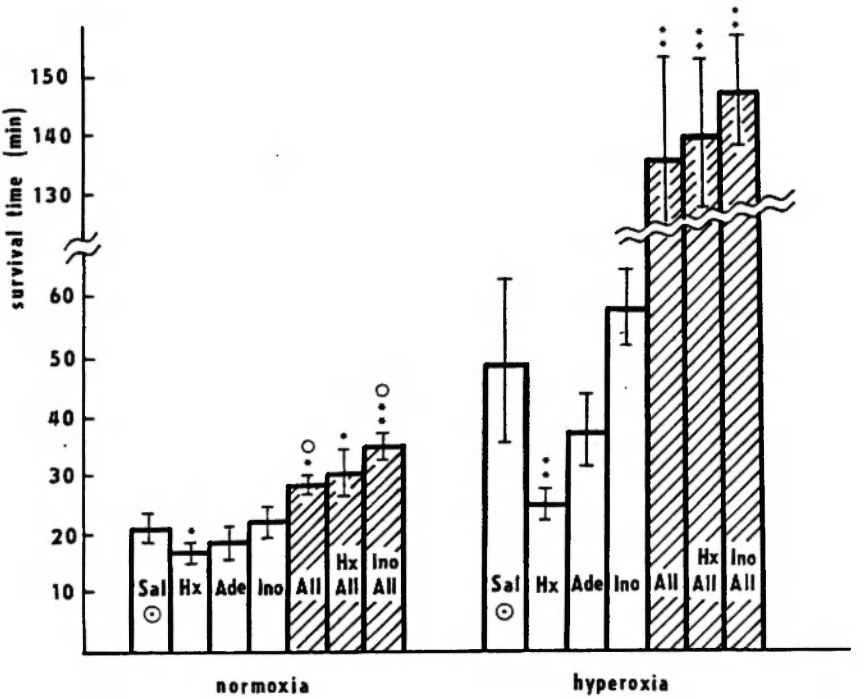

Fig. 1: Influence of purines on suvival time of rats in hypoxia interrupted by normoxia and/or hyperoxia. Sal - saline; Hx - hypoxanthine; Ade - adenine; Ino - inosine; All - allopurinol. Hatched columns: xanthine oxidase inhibited by allopurinol. Student's t-test: *$p<0.05$; **$p<0.01$; o$p<0.05$. Asterisks: Comparison to the appropriate control (Sal). Circles: Comparison inside the allopurinol group.

3. Comparison of XOD activities in the plasma of four animal species and their sensitivities to interrupted hypoxia

The higher the normal XOD activity in the plasma, the less resistant was the species to hypoxia (Table 1).

4. Effect of ethanol

The administration of ethanol resulted in a significant increase of XOD activity in the plasma of both rats and hamsters (Tables 2 and 3).

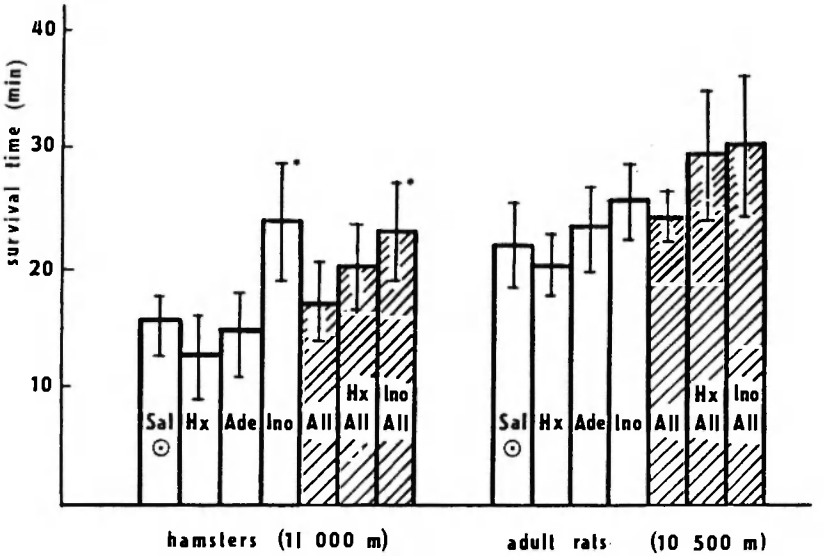

Fig. 2: Influence of purines on survival time of rats and hamsters in continuous lethal hypoxia. For symbols see Fig. 1.

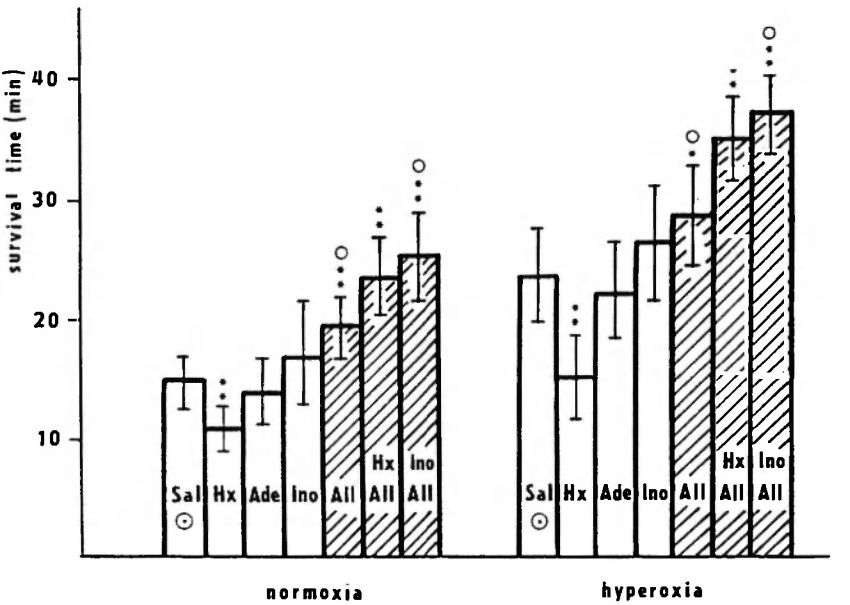

Fig. 3: Influence of purines on survival time of hamsters in hypoxia interrupted by normoxia and/or hyperoxia. For symbols see Fig. 1.

TABLE 1
Survival time in hypobaric hypoxia

Species	Normal Plasma Xanthine Oxidase (nkat/l)	Survival Time (min)
Guinea Pig	no measurable activity n=11	100.80±13.84 n=10
Hamster	no measurable activity n=10	54.25±11.33 n=20
Rat	99.46±13.85 n=20	25.20±5.37 n=10
Mouse	259.67±58.23 n=19	3.33±1.00 n=17

means±S.D.

TABLE 2
Ethanol-induced changes in rat plasma xanthine oxidase activity (nkat/l)

Time After Treatment (min.)	0	90	120
Controls (saline)	178.1±30.7 n=3	162.8±33.8 n=5	192.1±44.4 n=4
Ethanol (8 ml/kg)	267.9±60.3 n=5	334.0±68.0** n=5	411.7±65.3** n=3
Ethanol (12 ml/kg)	216.6±92.3 n=5	313.6±95.1** n=5	322.8±84.2* n=5

*p < 0.05; **p < 0.01 as compared to controls

TABLE 3
Ethanol induced changes in hamster plasma xanthine oxidase activity (nkat/l)

Time After Treatment (min.)	0	90	120
Controls (saline)	38.7±16.9 n=9	61.4+11.9 n=9	66.3±25.5 n=8
Ethanol (12 ml/kg)	58.6±14.7* n=9	105.6±32.7* n=9	116.1±28.4 ** n=10

*p < 0.05; **p < 0.01 as compared to controls

DISCUSSION

Xanthine oxidase is a membrane-associated and cytosolic enzyme /12/. High activity has been observed in the liver, intestine and kidney, and also in blood plasma. There is no correlation between plasma and organ activity; an interspecies variability has been described in organ XOD /13/. The native form of XOD seems to be the

D-form /14/. The conversion of about 30% of organ XOD to its superoxide producing the O-form has been described after ischemia /15/. The native form in plasma is the O-form – therefore, this activity can be involved in posthypoxic tissue damage.

The effect of hypoxanthine and allopurinol was obvious only in pre-exposed groups. Pre-exposure did not affect the survival time in the following lethal hypoxia (Figs. 1, 2, 3), but increased oxypurines in body fluids /16/. A mild hypoxic pre-exposure can also facilitate the conversion of the D-form into the O-form.

Hypoxanthine was more efficient when combined with hyperoxia rather than nomoxia. Thus, the toxic effect of hypoxanthine is moderated by Po_2 decrease and is not fully displayed until reoxygenation. The observed protective effect of allopurinol is in agreement with this assumption.

XOD reaction and its inhibition by allopurinol may affect resistance by a variety of mechanisms. Tissues may be damaged during interrupted or alternating hypoxia by reactive species of oxygen formed during the XOD reoxidation, as in the case of ischemia.

Another possible mechanism of the beneficial effect of allopurinol may be the protection of the purine pool from degradation. As a consequence of XOD inhibition by allopurinol, the increased quantity of hypoxanthine after hypoxia cannot be oxidised to uric acid and is used for the regeneration of adenine nucleotides via the "salvage pathway". Our experiments show the negligible effect of the stimulated salvage pathway (i.e., the administration of allopurinol simultaneously with hypoxanthine or inosine) compared to the decrease of survival time caused by free radical production after a hyperoxic break.

In rats, the protective effect of allopurinol was more obvious than in hamsters. This fact is in agreement with the negative correlation between the XOD activity in plasma of different species and the resistance of the species to alternating hypobaric hypoxia.

In our previous work we showed a correlation between the development of XOD in rat plasma and the increased sensitivity to hypoxia during ontogenesis /16/.

Hypoxia does not influence XOD activity in plasma /16/.

The administration of ethanol in our experiments increased the plasma XOD activity without elevation of any other enzymes. Increased lipid peroxidation and formation of reducing equivalents and acetaldehyde occurs due to ethanol metabolism via alcohol dehydro-

genase and via the microsomal ethanol-oxidizing systems. These products damage cellular membranes /17/; this may be associated with the release and conversion of XOD. Our finding af a small amount of D-form XOD in rat plasma is in agreement with this assumption (Novák, Štípek, Zima - in preparation).

CONCLUSIONS

1. Xanthine oxidase plays an important role in post-hypoxic tissue damage. Its activity in plasma can affect the resistance of the species to alternating hypoxia.

2. The protective effect of allopurinol is caused by the decrease of reactive oxygen species production. The stimulation of the salvage pathway by allopurinol and simultaneous application of other purines is negligible.

3. The plasma XOD seems to be a sensitive marker of liver damage. Its higher activity due to acute ethanol intoxication in the presence of appropriate substrates (oxypurines) may improve the ethanol organ damage.

REFERENCES

1. Deuticke B, Gerlach E, Diecksmann R. Abbau freier Nukleotide in Herz, Skeletmuskel, Gehirn und Leber der Ratte bei Sauerstoffmangel. Pflügers Archiv 1966; 292: 239-254.
2. Batelli MG, Della Corte E, Stirpe E. Xanthine oxidase type D (dehydrogenase) in the intestine and other organs of the rat. Biochem J 1972; 126: 747-749.
3. Fridovich I. Quantitative aspects of the production of superoxide anion radical by milk xanthine oxidase. J Biol Chem 1970; 245: 4053-4057.
4. Frank L, Massaro D. Oxygen toxicity. Am J Med 1980; 69: 117-126.
5. Cunningham SV, Keaveny TV, Fitzgerald P. Effect of allopurinol on tissue ATP, ADP and AMP concentrations in renal ischemia. Br J Surg 1974; 61: 562-565.
6. Gardner TJ, Stewart JR, Casale AS, Downey JM, Chambers DE. Reduction of myocardial ischemic injury with oxygen derived radical scavengers. Surgery 1983; 94: 423-427.
7. Saugstad OD. Hypoxanthine as a measurement of hypoxia. Pediatr Res 1975; 9: 158-161.
8. Al Khalidi UAS, Chaglassian TM. The sensitivity of serum xanthine-oxidase and serum glutamic pyruvic transaminase in detecting liver damage. Clin Chim Acta 1966; 14: 833-839.

9. O'Brien JJ, Lucey EC, Snider GL. Arterial blood gases in normal hamsters at rest and during exercise. J Appl Physiol 1979; 46: 806-810.
10. Saugstad OD. The determination of hypoxanthine and xanthine with a Po_2 electrode. Pediatr Res 1975; 9: 575-579.
11. Goldbaum LR, Domanski TJ, Schloegel EL. Analysis of biological specimens for volatile compounds by gas chromatography. Forensic Sci 1964; 9: 63-67.
12. Villela GG, Mitidieri E, Alfonso OR. Intracellular distribution of xanthine oxidase in the rat liver. Nature 1955; 175: 1087-1090.
13. Al Khalidi UAS, Chaglassian TM. The species distribution of xanthine oxidase. Biochem J 1965; 97: 318-320.
14. Stirpe F, Della Corte E. The regulation of rat liver xanthine oxidase. J Biol Chem 1969; 244: 3855-3863.
15. Akizuki S, Yoshida S, Chambers DE, Eddy JL, Parmley LF, Yellon MD, Downey JM. Infarct size limitation by the xanthine oxidase inhibitor, allopurinol, in closed chest dogs with small infarcts. Cardiovasc Res 1985; 19: 686-692.
16. Štípek S, Novák L, Vinklár P, Trojan S, Štastny F. Plasma xanthine oxidase correlates with the resistance to severe hypoxia in different species. Physiol Bohemoslov 1988; 37: 107-113.
17. Lieber CS. Biochemical and molecular basis of alcohol-induced injury to liver and other tissues. New Engl J Med 1988; 319: 1639-1650.