

ANTIHYPOXIC ACTION OF 5-PHENYLPROPYNYLIDENE RHODANINE DERIVATIVE IN HYPOXIA-INDUCED RAT

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Hypoxia, which is inadequate of tissue oxygen supply. Investigation of Antihypoxic activity in two models of experimental hypoxia i.e. haemic hypoxia and circulatory hypoxia. In addition, several studies suggest that thiazolidine derivative improve of oxygen supply to tissues under condition of its deficiency and decrease of incompletely oxidized product of metabolism. Highly resistant and low resistant rats divided by their individual resistance to hypoxia. Pathological process developing in animals placed under the vacuum bell jar was made with exposure time 60 min. Medicinal agents (5-phenylpropynylidene rhodanines) was introduced and after modeling pathology in dose 1/10 LD₅₀. The control group of animals was subjected without introducing of medicinal agents. Content of nitrite-ion in serum and in homogenate was determined applying methods. Reduction of nitric oxide synthesis was observed in liver hypoxic hypoxia. This can be accompanied by increase the level of lipoperoxidation processes and by suppression of antioxidant activity and mitochondrial enzymes. Furthermore the study indicated that hypoxia is inadequate of tissue oxygen supply, there was number of drugs for treatment of hypoxia such as circulatory hypoxia and haemic hypoxia. Evaluating the mechanism of 5-phenylpropynylidene rhodanine derivatives should show some potential action of antihypoxic activity.

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1. Introduction

Hypoxia, which is lack of oxygen at the tissue level like hypoxemia i.e. lack of oxygen in the blood that is low arterial oxygen content (Cao₂). In aquatic ecosystems, low oxygen usually means a concentration of less than 2-3 milligrams of oxygen per liter of water (mg/l). A complete lack of oxygen (0 mg/L) is called anoxia [1].

The fundamental purpose of the corresponding system is to deliver oxygen in the cells and to remove carbon dioxide from them. Proper maintenance of this function depends on intact cardiovascular, respiratory system and a supply of inspired gas containing adequate oxygen. The ideal hypoxia detection technique would be minimally or noninvasive, independent of cell or tissue type and nonoxygen substances, display a high contrast between air and nitrogen, and be able to monitor variations in oxygen levels over the smallest distances expected to contain oxygen gradients. Of the many different techniques that are currently being developed [2, 3]. The pathological mechanism for brain ischemia-hypoxia injury relates to excitotoxicity of excitatory amino acids (EAA), overload of intracellular free calcium and oxygen free radicals, which are not independent and ordinal, but associated with and enhanced by each other [4]. And it is suggested

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that excessive oxygen free radicals play a pivotal role in ischemia-hypoxia injury, especially in the period of reperfusion or reoxygenation [5]. The disorganized and dysfunctional tumor vasculature is generally attributed to this deficiency in oxygen supply. As we aimed to study the effect of enhanced oxygenation (“the flip of the coin”) using hyperbaric oxygen (HBO) treatment in a rat hypoxic hypoxia [6-8].

Hypoxia resulting from a defective mechanism of oxygenation in the lungs as caused by a low tension of oxygen and abnormal pulmonary function airway obstruction or right to left shunt in the heart [9]. When oxygen is in solution, it can more easily reach tissue areas where blood cells cannot pass, and can also enable tissue oxygenation even with impaired hemoglobin carriage [10]. Investigation of antihypoxic activity in two models of experimental hypoxia i.e. haemic hypoxia and circulatory hypoxia [11]. 4-thiazolidine is also modulate with the help of transformation in position and using reaction of acylation, heterocyclization which have been performed pharmacological screening of compound, antioxidant, anticonvulsive, anti-inflammatory, antitumoral, anti-ischemic, antihypoxic and antimicrobial activities was studied. Thiazolidine derivative having antihypoxic property, mechanism by which thiazolidine diones act with activation of peroxisome proliferators activated receptor (PPAR α). The anti-inflammatory activity of thiazolidine derivative is more pronounced than NSAID or the therapeutic index of this derivative uniformly been shows to be superior to that of classical NSAID. Some NSAID shows antihypoxic property because of that some thiazolidine derivative like indomethacin that is improvement of oxygen supply to the tissue under condition of its deficiency [12]. Some thiazolidine derivative is effective against tumor hypoxia because different cell lines, which is selected by some human cancer (tumor cell growth) and inhibited by concentration dependent growth suppression of the leukemia cell lines, renal line, breast cancer cell line and glioblastoma tumor cell line [13]. Hepatic hypoxia occurs during liver surgery and transplantation. The critical level associated with irreversible hepatocellular damage is unknown. Measurement of hepatic tissue oxygenation and hepatic vein oxygen partial pressure [HVPO₂] reflects oxygen supply and consumption. Near infrared spectroscopy [NIRS] can be used to monitor hepatic oxyHb and deoxy Hb and cytochrome oxidase oxidation. This study compares regional hepatic tissue oxygenation (HBO₂, HB and Cyt Ox) using near infrared spectroscopy with HVPO₂. The use of tissue oxygenation measured NIRS and HVPO₂ as indicators of hepatic tissue hypoxia was also investigated [14]. In accordance with the invention, we have found that rhodanine derivatives are of a potent inhibitory activity against chemical mediators including leukotrienes.

Recently, it has been elucidated and given attention that SRS-A, i.e. Slow Reacting Substance of Anaphylaxis, which has been known for a long time to be one of important chemical mediators for immediate anaphylaxis or asthma, is a metabolite of arachidonic acid by 5-lipoxygenase pathway, that is a mixture of leukotrienes C₄, D₄ and E₄. Moreover, it has been suggested that histamines, leukotriene B₄, PAF of prostaglandins, e.g., PGF_{2 α} , PGD₂, TXA₂, in addition to SRS-A, could participate in allergy or inflammation.

2. Experimental

2.1 Drug Synthesis

Derivative of 5-phenylpropynylidene rhodanine was synthesized, from cinamic aldehyde and rhodanine-3-hexamine acid by crystallization with diethyl formamide in presence of methanol formation of sodium salt of 5-(phenylpropynylidene)-3-carboxypentyl-2 thioxo-4 thiazolidinedione. Molecular weight-383.47

2.2 Experimental animals

Wistar rats weighing 180-200 g were used in this study. All animals were fed the same diet and maintained in a constant environment with a 12:12-hour light dark cycle. The objective of investigation were divided by their individual resistance to hypoxia i.e. high resistance (HR) and low resistance (LR).

2.3 Experimental procedure

The rats were randomly divided into two groups according to using the method described by V.A.Berezovsky (1975). Each rat was placed under the vacuum bell-jar where partial pressure of oxygen was created incompatible with life threatening (30 mm of mercury column), which corresponds to altitude 12,000 m above the sea level. Elevation to such altitude was made with velocity 200 m/sec during 1 min. The time of survival of the animals under vacuum till commencement of convulsions was taken as the criteria of the animal's resistance. Pathological process was developed in the animals placed under the vacuum bell-jar where the model of hypoxia (6,000 m above the sea level) was made with exposure time 60 min. served as the experimental model. Medicinal agent (derivative of 5-phenylpropynylidene rhodanines) was introduced by per oral after modeling pathology in dose 1/10 LD₅₀. Medicinal agent and arginine (modulate consumption of oxygen) was introduced to another group.

The control group of animals was subjected to the extreme effect without introduce of the medicinal agent (derivative of 5-phenylpropynylidene rhodanines). Homogenate of the liver tissue of rats and blood serum was used.

2.4 Estimation of lipid peroxidation and nitric oxide

Detection of the level of lipid peroxidation (malondialdehyde MDA) in rat liver tissue and content of nitrite-ion (nitric oxide NO) in plasma and homogenate liver tissue was determined by Ohkawa et. al method [15].

2.5 Statistical Analysis

Experimental values are means±S.D. of the number of experiments indicated in the legends. Data were evaluated for statistical significance with One-way ANOVA followed by Tukey's post hoc test. P<0.05 was defined as the level of statistical significance. The analyses were performed with the Graph-Pad InStat statistical package (Graphpad Software Inc.).

3. Results and discussion

3.1 Lipid peroxidation in rat liver tissue

In hypoxia content of oxygen radicals become increased, but becomes normal after return to normoxia. We found that the mean compared with high resistance control group was significantly decreased in the medicinal agent group (P <0.05) and medicinal agent + arginine group (P <0.05).

However, the low resistance control group was significantly higher in the medicinal group (P<0.05) and medicinal agent + arginine group (P<0.05). Results are shown in Tab.1

Table 1. Lipid peroxidation in rat liver tissue (malondialdehyde MDA)

Control		Medicinal agent		Medicinal agent + Arginine	
HR	LR	HR	LR	HR	LR
350.6±25.5	124.3±15.8	271.4±19.7*	167.8±20.8*	203.3±18.4*	211.3±21.8*

HR: High resistant, LR: low resistant. Values are expressed as mean±S.E.M ($n=6$). Values are statistically significant at * $P<0.05$ vs. control group.

3.2 Nitric oxide in plasma and in liver tissue under experimental conditions

In observation it is known, that initial response of microbloodstream to hypoxia was accompanied by the formation of active oxygen metabolites with protective effect of NO observed.

Compared with the control group, NO decrease in the medicinal agent group ($P < 0.05$) and medicinal agent + arginine group ($P < 0.05$). Although the regulation of NO production by the cells in response to hypoxia may take place on the levels of transcription, expression of enzyme, modulation of its activity, presence of substratum as well as through the mechanism of the system level and the organism in general. We found that, significant increases were observed in the control group compared with the medicinal agent and medicinal agent + arginine group ($P < 0.05$). Results are shown in Tab.2

Table 2. Nitric oxide in plasma under experimental conditions

Control-plasma level	NO plasma level, 400mg/kg (P.O)	NO plasma level, 400mg/kg (P.O)
13.58±0.69	N=5;46±0.8*	N=4;11.1±0.45*

NO: Nitric oxide, PO: Per oral, N-number of animals. Values are expressed as mean±S.E.M ($n=6$). Values are statistically significant at * $P<0.05$ vs. control group.

In contrast, the relative levels of medicinal agent and medicinal agent + arginine group ($P < 0.05$) were significantly lower in the plasma and liver tissue. Results are shown in **Tab.3**

Table 3. Nitric oxide in liver tissue under experimental conditions

Control- LTL	NO - LTL, 400mg/kg (P.O)	NO - LTL, 400mg/kg (P.O)
45.73±1.13	N=5; 35.28±0.86*	N=4; 34±0.18*

NO: Nitric oxide, LTL- liver tissue level, PO: Per oral, N-number of animals. Values are expressed as mean±S.E.M ($n=6$). Values are statistically significant at * $P<0.05$ vs. control group.

The level of transcription mRNA and NOS may not coincide with the level of expression of this protein in acute hypoxia, but may create the possibility of further induction of protein-enzyme and NO synthesis depending on the intensity of the effect and the time of development of response to it[16].

Induction of protein and NOS as well as the varying speed and degree of enzyme induction in various organs and tissues depend on the length of acute hypoxia, which points at the

successive inclusion of the organs and systems in NO-dependent compensatory mechanism in hypoxia. Under the conditions of hypoxia arginine which helps to provide oxygen in proper way and in blood utilized with the formation of nitric oxide and its products of metabolism-NO₃⁻; NO₃ anions [17]. Nitrite anions ensure acceptance of electrons by cytochrome oxidase under the hypoxic conditions. The activation of the cycle of nitric oxide which was the effective product of its metabolism-nitrates and nitrites for energy wise economical reactions connected first of all with functioning of cGMP [18]. The increase of cholinergic regulatory effects with participation of cGMP causes reduction of tissues for oxygen and the intensity processes of lipoperoxidation [19, 20]. We investigated that the reduction of the synthesis of nitric oxide was observed in the liver hypoxic hypoxia. This could be accompanied by the increase of the level of the processes of lipoperoxidation and by the suppression of activity of antioxidant and mitochondrial enzymes [21, 22].

4. Conclusions

The actuality of problem of hypoxia that given very deleterious affects on cardiovascular system that hypoxia may worsen the condition of myocardial infarction and central nervous system disorder such as hypoxic ischemic encephalopathy and also blood circulatory system eg. circulatory hypoxia etc.

Our study indicated that there are number of medicine now a day available for treatment of hypoxia i.e. drug having remarkable effect on different types of hypoxia such as circulatory hypoxia, anemic hypoxia and respiratory hypoxia etc. Yet some group of drugs most helpful in the treatment of hypoxia such as excitatory amino acid antagonist (EAA), calcium channel blockers, some anti-inflammatory drugs eg. Indomethacin and notropic agent, drugs use in seizures i.e. Diazepam. Again we discussed a drug that is thiazolidine derivative eg. 4-thiazolidone should show some potential as antihypoxic activity i.e. 5-phenylpropynylidene rhodanines.

List of abbreviations

PPAR α -Peroxisome proliferators activated receptor, NSAID- Nonsteroidal anti-inflammatory drug, HVPO₂ –Hepatic vein oxygen partial pressure, IR- Infrared spectroscopy, Cyt Ox- Cytochrome oxidase, SRS-A- Slow reacting substance of anaphylaxis, NO- Nitric oxide, EAA- Excitatory amino acid antagonist.

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References

- [1] Ecological Society of America.
- [2] H.B.Stone, M.J.Brown, T.L.Phillips, R.M.Sutherland, Radiat.Res. **136**, 22(1993).
- [3] Assessment of tissue hypoxia: Jukka Takala, European society of Anesthesiologists (2000).
- [4] P.A .Wilkins: Hypoxic ischemic Encephalopathy: 16 December 2003.
- [5] P.H.Chan: Role of oxidants in ischemic brain damage, Stroke. **27**, 1124 (1996).
- [6] Hepatocellular hypoxia induced vascular endothelial growth factor expression and angiogenesis in experimental biliary cirrhosis: American Society for Investigative Pathology (American Journal of Pathology 1999).

- [7] E.Stokebaum, D.Lambrechts, Carmeliet :VEGF: once regarded as a specification of angiogenic factor now implicated neuroprotection.(National library of Medicine, Bioassays; 2004)
- [8] J. L.Boulnois, J. Pechoux, JCMC: Hemosonic (Elements of Heamodynamic Monitoring) Arrow International.16 (2000).
- [9] D.J. Pierson MD, FAARC, Respire: Pathophysiology and clinical effect of chronic hypoxia (2000).
- [10] D. M. Brizel, S. Lin, J. L. Johnson, J. Brooks, Dewhirst, et al.,The mechanisms by which hyperbaric oxygen and carbogen improve tumor oxygenation. Brit J of Cancer. **72**, 1120 (1995).
- [11] R. B. Lesyk, B. S. Zimenkovsky, Thiazolidine; centenarian history, current status and perspective for modern organic and medical chemistry. Dept of pharmaceutical chemistry Live national Medical University Ukraine.
- [12] A.T.Berezoniakova, V.M.Kuznetsova: The mechanism oh antihypoxic action of indomethacin, diclofenac and ibuprofen.
- [13] N.C.Denko, A.J.Giaccia: Tumor hypoxia, the physiological link between Trausseau's Syndrome and metastasis, Stanford University, School of Medicine, California. Cancer res. (2001).
- [14] A.E.H. El-Desoky, Jiao LR, Havlic R: Measurement of hepatic tissue hypoxia using near infrared spectroscopy: Comparison with hepatic vein oxygen partial pressure. (E.S.R. 2000).
- [15] H.Ohkawa, N.Ohishi , K.Yagi : Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochem. 95 , 351–358 (1979).
- [16] Lucia de Tranceschi, A.Baron, A.Scarpa, P.Rouyer – Fessard: Inhaled nitric oxide protects transgenic SAD mice from sickle cell disease specific lung injury induced by hypoxia / reoxygenation (April 2003).
- [17] G.C.Brown, N.Foxwell and S.Moncada : FEBS Lett. **439**, 321–324 (1998).
- [18] L.Paakkari, P.Lindsbergs: Nitric oxide in the central nervous system: Ann. Med **27**, 369 (1995).
- [19] E.H.Cao, X.Q.Liu, J.J.Wang & N.F.Xu : Biochim Biophys Acta. **1259**, 187 (1995).
- [20] H.B.Demopoulos, E.S.Flamm, D.D.Peitrongro : The free radical pathology and the microcirculation in the major central nervous system disorders, Acta Physiol. Scand 492- 91, (1980).
- [21] A.Bal-Price and G.C.Brown : J. Neurosci. **21**, 6480 (2001).
- [22] M.C.Longuemare, M.P.Hill and R.A.Swanson : Neuroreport. **5**, 1789 (1994).