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Importance of Amaranth Oil for Protection against Hypoxia.

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ABSTRACT

The article deals with squalene, which is considered to be an important intermediate of cholesterol synthesis and a vegetable terpenoid, and stated to demonstrate hypolipidemic, antiatherosclerotic, immunotropic, antitumoral and radioprotective activity in animal models, in vitro environment and in humans experiments. Significant amount of squalene is present in the livers of sharks; in flora it can be found in olive amaranth and palm oils, wheat germ oil and brown rice oil. Amaranth oil also appears to be a source of unsaturated fatty acids, polyphenols and an active form of vitamin E. The aim of this study was to reveal squalene antihypoxic activity depending on the presence of other natural antioxidants in various models of experimental hypoxia in rats. 14-day oral squalene administration, daily dosage 0.05g per one animal, was estimated to prolong life time and decrease mortality. Antihypoxic activity was revealed in three models of hypoxia. Significant differences between groups receiving squalene as a part of amaranth oil diet and squalene complemented to refined corn oil in the lack of other natural antioxidants were not revealed. Thus, a diet rich in squalene is supposed to be effective in the complex therapy of diseases associated with hypoxia of various origins.

Keywords: amaranth, antioxidant, diet, experiment, life time, oil, squalene.

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BACKGROUND

Squalene appears to be a carbohydrate of a triterpene series – the basic non-steroid intermediate of the cholesterol biosynthesis. In animals squalene is contained in large amounts in the liver of deepwater sharks (*Squalus spp*.) [1]. Its significant amounts have been found in olive, palm, amaranth oils, wheat germ oil and brown rice oil. Squalene presenting in the human body may result from the synthetic reactions at the stage of cholesterol formation or enter with food. Approximately 60-85% of squalene obtained with food is absorbed and reaches blood plasma [2], mainly together with very low- density lipoproteins.

Currently there are data on various types of squalene biological activity. Thus, squalene in combination with pravastatin and other HMG-CoA inhibitors normalizes triglucerides level in plasma, effectively decreases cholesterol level, mainly due to the increased faecal elimination of the latter [3]. Squalene is considered to be one of the most important components of lipid mantle acting as a trap of free radicals and protecting human skin from harmful impact of UV rays and photo ageing [4]. Squalene plays a similar role in the eye retina of the vertebrates protecting photoreceptors from free-radical damage [5].

In literature there are data that squalene increase in a diet allows stimulating the reticuloendothelial system [6], enhancing cellular immunity and non-specific immune defense in a dose-dependent manner [7-9].

Radioprotective action of squalene is supposed to be realized through decrease of leukocytes death and increase of lifetime of experimental animals [10].

One of the most widely discussed effects of squalene is its onco-protective effects. Squalene has been stated to restrain the growth of tumor cells and partially prevent the development of chemo-induced cancer [11, 12, 13]. It has been demonstrated on experimental models that squalene potentiates cytotoxic activity of certain chemotherapeutic preparations [14] and may result in regression of certain tumors [15], at the same time protecting a white blood lineage in cisplatin- and carboplatin-induced damage of tumor cells [16].

In long-term administration squalene is able to normalize cholesterol and lipoproteins levels [17, 18].

A wide range of biological activity and relatively low toxicity gives an opportunity to consider squalene as an additional dietary factor in various diseases. Successfulness of such an approach has been proved by the so called “Mediterranean diet” rich in unsaturated fatty acids, phytosterols and flavonoids [19, 20, 21, 22, 23]. This approach allows reducing risk of cardio-vascular diseases, diabetes, obesity, oncological diseases; it is important for hemostasis regulation and an immune response. Olive oil containing a large amount of monounsaturated fatty acids, polyphenols, tocopherol and squalene is reported to be one of the most important integral components of the Mediterranean diet.

Amaranth oil is characterized by high squalene content [17]; its concentration may reach up to 9% in various techniques of production. Except squalene, it also contains a large amount of monounsaturated fatty acids, vitamin E in an active tocotrienol form.

High antioxidant activity of squalene allows assuming the fact that in certain acute and chronic disorders, whose pathogenesis is tightly related to free radicals formation, preventive administration of squalene as a dietary component may significantly reduce the level of their harmful impact. Hypoxic conditions are known to be accompanied by a transit into an anaerobic way of oxidation and release of a large amount of free radicals. At the same time, hypoxia appears to be a complicated multifactorial pathologic condition, whose mechanisms are not limited by the generation of free radicals only. Correction of hypoxic conditions is, undoubtedly, one of the actual challenges of modern medicine.

The aim of this study was to reveal experimentally the dependence of squalene anti-hypoxic activity on the presence of vitamin E in hypoxic models.

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MATERIALS AND METHODS

Drugs

Amaranth oil produced by OOO "RusOliva" with cold pressing method was used for feeding of experimental animals. To prepare solutions with various squalene amounts the authors used food refined corn oil as a transport and squalene, produced by **"SIGMA-ALDRICH"** Co, St. Louis, MO, USA (Specific gravity: 0.858)**.**

Animals

Experiments were performed on white outbred male rats at the Pharmacology Department of the Voronezh N.N. Burdenko State Medical University. 82 male rats weighed 180-220 g and 144 male mice weighed 18-20 g were used in the experiments. Animals were randomly housed in groups of five in a temperature-controlled environment (22 ± 2°C) under a 12-h light/dark cycle, with *ad libitum* access to food and water except during experimental procedures. The experimental protocol was approved by the Voronezh State University Ethics Committee.

Drug treatment

Animals were divided into 8 groups, 9-12 species each, for each type of hypoxia. Animals of the control group (C) did not receive anything except standard vivarium ration (keeping ration: protein 19%, fats 5%, carbohydrates 7%, 3100kcal/kg of feed). Animals of the experimental groups received oils with various squalene amounts once a day during the whole period of the experiment by forced feeding through a gastric tube for 14 days. The amount of introduced oil was similar in all groups and constituted 3.9 ml/kg.

Animals of the control group (CO) were introduced 3.9 ml/kg of refined corn oil per orally. In experimental series (groups A1, A2, A3) 3.9 ml/kg of amaranth oils with squalene concentration 6%, 3% and 1.5% respectively, and squalene oil solution on the refined corn oil (groups C1, C2, C3) with squalene concentration 6%, 3% and 1.5% was introduced.

Anti-hypoxic effect was studied on three models of hypoxia. Hypoxic hypoxia was simulated by placing rats in a hermetic chamber 1.5 liter-capacity and raising through a certain height – a "death zone" defined in the preliminary series of experiments; there was measured life duration at a height, survival rate and protection factor (PF) calculated according to the formula $PF=(a/b+1)(c/d+1)$, where "a" and "b" – number of animals survived in the experiment and control, "c" and "d" – total amount of animals. Histotoxic hypoxia was simulated by subcutaneous introduction of sodium nitroprusside dosage 25 mg/kg to mice. Acute hematic hypoxia was simulated by intraperitoneal introduction of sodium nitrite dosage 225mg/kg to mice. Life duration of animals was specified.

Statistical analysis.

All results were expressed as the Mean ± S.D. for seven animals in each group. All the grouped data were statistically evaluated with SPSS 10.0 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test; significance level at $p < 0.05$ was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Hypoxia is a pathological condition associated with numerous acute and chronic disorders. Pathogenesis of human hypoxic conditions is rather complicated and is not described completely by any known method of simulating experimental hypoxia on animals. That is why, it is recommended to use several models of hypoxia when developing anti-hypoxic preparations, regimens of their application or ways to reduce negative hypoxic effects, to prove their efficiency [24]. Antihypoxic drugs are applied in the treatment of the central nervous system disorders, in cardio-vascular pathology and others. Antihypoxic activity of amaranth oil and flour in the model of hypoxic hypoxia was registered in some works [25, 26]; this allows assuming, to a certain extent, an opportunity to apply amaranth oil rich in squalene as a dietary component in patients with various diseases, where hypoxia appears to be the leading pathogenetic element. We consider it important not only to record the occurrence of anti-hypoxic activity of a squalene-containing diet, but to specify the dependence of squalene anti-hypoxic effect on the regimen of application and presence of other natural nutrients – components of amaranth oil.

Presence of the control group receiving refined corn oil was determined by the necessity to provide equal nutritional conditions in the control and experimental series, thus, excluding any influence of transport corn oil on the results of the experiment.

We have determined that amaranth oil in the described regimens, in the same way as squalene, has a significant antihypoxic effect in three basic models of hypoxia of various genesis in the regimen of short-term course application due to essential amount of squalene, phospholipids, unsaturated fatty acids, fat-soluble vitamin E. The highest activity was observed on the model of hypoxic hypoxia, as Table 1 demonstrates. Hypoxic hypoxia is simulated by placing animals in a hermetic chamber, where oxygen amount in the inhaled air is significantly lower than in their habitat.

Table 1: Hypoxic hypoxia (amount, % of dead animals by the end of the experiment) at the 30th minute of observation

Model /group	Control	Corn oil	A1	A2	A3	C1	C2	C 3	всего
Squalene amount, %	0	0	6	3	1,5	6	3	1,5	
Letality (% of dead)	66,7	70	27,3*	55,6	66,7	30*	45,5	50	
Protection factor		0,55	1,57	1,14	1	1,5	1,3	1,23	

*statistically significant results in comparison with the control (p≤0,05)

Table 2: Life duration (minutes) in the models of histotoxic and hemic hypoxia

GROUP	Hystotoxic hypoxia, M± m	Hemic hypoxia, M± m
Control	9,83 ± 0,14	12,35 ± 0,4
Corn Oil	8,8 ± 0,48	11,6 ± 0,45
A1	29,28 ±0,69*	20,4 ± 0,14*
C1	31,2 ±0,47*	21,3 ±0,24*

*statistically significant results in comparison with the control (p≤0,05)

Thus, mortality in the groups A1 and C1 with the highest squalene doses constituted 27 and 30% respectively, whereas in the control group and in the group receiving transport oil the mortality rate was 67-70%, p≤0,05. When modeling hypoxic hypoxia in groups A1-A3 receiving amaranth oil, there was registered a tendency to increased life duration of animals and protection factor in comparison with groups C1-C3 receiving similar doses of squalene but in the refined corn oil.

Histotoxic (tissue) hypoxia results from the reduced or lost tissue ability to utilize oxygen; it develops in case of sleeping pills overdosage, e.g. barbiturates. In the experiment tissue hypoxia is simulated by nitroprusside introduction. Hemic hypoxia results from the reduced amount of erythrocytes, their massive damage or decrease of functionally active blood hemoglobin. Experimental hemic hypoxia is simulated by sodium nitrite introduction. In the models of histotoxic and hemic hypoxia life duration increased proportionally to the increased squalene dose. However, statistically significant differences in comparison with the control was registered only in groups A1 and C1, with squalene amount 6% of the introduced oil volume. An expressed tendency to increased life duration was registered in groups with lower squalene amount; however, it appeared to be invalid in p≤0,05.

Amaranth oil received by cold pressing is known to contain a large amount of biologically active substances able to independently influence the course of hypoxic conditions – triglycerides, phospholipids, phytosterol, tocopherols, carotinoid, unsaturated fatty acids. At the same time, no statistically significant differences were observed between the efficiency of the same concentrations of squalene diluted in the refine corn oil and squalene surrounded by unsaturated fatty acids, tocotrienol vitamin E and other natural antioxidants of amaranth oil in short-term oil application with squalene amount from 1.5 to 6%. A tendency to increased life duration in groups receiving 3 and 1.5% squalene of the introduced oil allows assuming that longer application of a squalene-containing diet with a small squalene amount may also appear to be effective in preventing hypoxic conditions.

Squalene antioxidant activity is determined by its ability to trap free radicals [27, 4]. It is known that formation of reactive oxygen intermediates may have either regulatory (nitrogen oxide NO and superoxide OO) or harmful significance (OH^*; lipoxylic radical LO^*, peroxynitrites) in relation to membrane lipids, DNA molecules, carbohydrates and proteins and accompanies practically any pathological process in the human body [28, 29]. Comorbid conditions, age, constant stress, unhealthy lifestyle and, by far not the last thing, irregular diet may result in balance disorder between free-radical load and tension of the body antioxidant systems.

To summarize all the above mentioned, we may assume that nutritional squalene plays an important role in the diet of patients suffering from atherosclerosis, ischemic heart disease, hypertonia, diabetes [30, 31, 32, 33] and others, where activation of lipid peroxidation contributes to pathogenesis. To finally answer the question on the significance of squalene in the treatment of diet-dependent diseases it is necessary to continue investigations in the given area.

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