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Influence of Dihydroquercetin, 1-Aryltetrahydroisoquinoline (F-18) and Their Conjugate (DHQ-11) on Mitochondrial Respiration and Oxidative Phosphorylation

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Abstract: In current scientific article, rat liver mitochondria respond to the flavonoids dihydroquercetin (20–100 μM), 1-(2′-bromo-4′,5′-dimethoxyphenyl)-6,7-dimethoxy-1,2,3,4 for respiratory and processes of oxidative phosphorylation - tetrahydroisoquinolines F-18 (20-100 μM) and synthesized on the basis of followings 2-(3,4-dihydroxyphenyl)-6-[1-(2'-bromo-4',5'-dimethoxyphenyl)-6,7- The effect of dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl]methyl-3,5,7-trihydroxychroman-4-on the concentrations of the DHQ-11 conjugate (20-100 μM) was investigated in *in vitro* experiments. Experiments were conducted within 180-200 gram infertile white male rats. Mitochondrias from rat liver were isolated by the way of differential centrifugation. Concentrations of dihydroquercetin flavonoid 20, 60, 100 μM in FAD-dependent succinate oxidation in rat liver mitochondria were identified to be reliable respiratory control parameters and partially increased ADF/O value, the respiratory control coefficient as Chans demonstrated an increase in respiratory rate under the influence of the alkaloid F-18 isoquinoline 20, 60, 100 μM. Moreover, the ADP/O coefficients also boosted under the influence of the isoquinoline alkaloid F-18. It was observed that ADP/O ratio coefficients was expanded within the bonding by the effect of the DHQ-11 conjugate concentration. The concentration of 100 μM dihydroquercetin for FAD-dependent succinate oxidation in rat liver mitochondria surged Chance's respiratory rate by 15% compared to the control. Concentrations of isoquinoline alkaloid F-18 100 μM enlarged the respiratory rate by 13% compared to the control. It was defined that concentrations of 100 μM DHQ-11 conjugate rose the respiratory rate by 20%. The oxidation of FAD-dependent substrates in mitochondria was more actively effected by the DHQ-11 conjugate than dihydroquercetin and F-18 isoquinoline alkaloids.

Keywords: Liver, Mitochondria, FAD, ROS, Succinate, Dihydroquercetin, F-18 Isoquinoline Alkaloid, DHQ-11 Conjugate

1. Introduction

In today's day and age, according to the statistics of the World Health Organization, about 355 million people worldwide are diagnosed with liver disease, and this amount is growing year by year [7]. Indeed, this requires further research in this sphere properly. In this regard, it is crucial to identify compounds within a low level of side effects, high biological activity based on natural plant materials, as well as creation of new pharmacological preparations with cardioprotective and hepatoprotective effects.

Flavonoids belong to a large class of natural heterocyclic compounds. Flavonoids possess antioxidant, anti-inflammatory, anti-inflammatory, and cytoprotective properties [10, 14]. Therefore, flavonoids may interfere with

the activation of endogenous antioxidants and the formation of reactive oxygen species (ROS) [4]. Flavonoids can readily bind to free radicals resulting in a decreasing of free radicals [5]. The presence of OH groups in the structure of flavonoids ensures their high reactivity towards ROS and reactive nitrogen species (ROS) [3]. The antioxidant effectiveness of flavonoids may increase depending on the amount of OH in the molecule [13, 9]. The increase in free radical formation is associated with the function of electron transport in the mitochondrial respiratory chain, which can be manifested by inhibition or activation of the V_3 and V_4 respiration states [2]. Currently, there are huge amount of biological substances that regulate the interaction of oxidative phosphorylation processes that can effectively influence on the frequency of mitochondrial respiration, respiratory control and ADP/, increase of ATP synthesis [6]. Such biologically active substances include dihydroquercetin flavonoids and isoquinoline alkaloid F-18 (1-(2′-bromo-4′,5′-dimethoxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline 3,4 - synthesized on the basis of dimethoxyphenylethylamine and 2-bromo-4,5-dimethoxybenzaldehyde) [15] and the biological activity of the DHQ-11 conjugate based on them were investigated deeply [8, 12]. However, the effects of these compounds on the level of liver mitochondria have not been identified in *in vitro* experiments.

For this purpose, in our present work, oxidative phosphorylation of the flavonoid dihydroquercetin [1] and isoquinoline alkaloid F-18 isolated from Siberian pine, as well as DHQ-11 conjugated compounds based on them, was oxidized by rat liver mitochondria.

The aim of the study was to investigate the effect of dihydroquercetin, isoquinoline alkaloid F-18, and a conjugate based on them (DHQ-11) on respiration and oxidative phosphorylation of rat liver mitochondria.

2. Materials and Methods

2.1. Experimental Animals

The experiments were carried out on sterile white rats weighing 180-200 gr.

2.2. Isolation of Rat Liver Mitochondria

Mitochondria were isolated from the liver of rats by the way of differential centrifugation. Rat livers were homogenized within a Teflon glass homogenizer and resuspended in 30 ml incubation medium: 250 mM sucrose, 1 mM EDTA and 10 mM Tris-HCl, 0.5 mg/ml BSA (Bovine serum albumin), pH 7.4. At the first stage, the liver homogenate was centrifuged at 2000×g for 10 min. The expected supernatant was obtained and centrifuged at 6000×g for 20 min. The mitochondria purified from the separation condition were collected by an autopipette into a special container. For the aim of conducting experiments, mitochondria were washed in a ratio of 1:5 without EDTA and stored in an ice container in a refrigerator.

2.3. Measurement of Mitochondrial Oxidative Phosphorylation

Respiratory rate and oxidative phosphorylation of liver mitochondria were determined by way of polarography. Oxygen consumption was measured using a Mitocell C 200 microrespirometric system based on a Clark-type oxygen electrode (Strathkelvin Instruments, Scotland). The rate of oxygen consumption by mitochondria is measured time by time, with the amount of added ADP being 200 μM and DNP being 5 μM. V_2 is the rate of mitochondrial respiration in substrates without ADP. V_3 is the rate of mitochondrial respiration activated by the addition of ADP to the environment. V_4 is the rate of mitochondrial respiration after the conversion of added ADP to total ATP. V_{DNF} is the maximum respiration rate in the presence of DNF, which separates oxidation from phosphorylation in the environment. Respiratory control V_3 was calculated as V_4 ha (V_3/V_4). The ADP/O value is calculated as the ratio of 200 μM ADP to the amount of oxygen (μg) utilized in the oxidative phosphorylation process. The experiments were carried out at room catching temperature (26°C) with constant stirring in a 0.5 ml cuvette. The amount of protein in the cell is formed for 1 mg/ml.

The rate of substrate oxidation in different metabolic states is given by O_2 μg/mL relative to the mitochondrial protein. The amount of protein in mitochondria was found by Peterson's modification of the Lowry method [11].

Statistical processing from the results were analyzed applying the Origin 8.6 computer program (OriginLab Corporation, USA).

3. Results and Discussion

In preliminary experiments, we investigated the effect of dihydroquercetin concentrations of 20, 60, and 100 μM on the oxidation and oxidative phosphorylation of FAD-dependent substrates in rat liver mitochondria (Figure 1). In the case of V_2, mitochondrial oxygen uptake was 7.38 μg atoms O_2/min. formed. Oxygen consumption in the case of dihydroquercetin 20, 60 and 100 μm at V_2 concentrations was 8.41 μg atoms O_2/min, 9.84 μg atoms O_2/min and 13 μg atoms O_2/min contributed. As a result of the concentration-dependent action of dihydroquercetin, oxygen consumption in V_2 increased compared to the control. By transferring 200 μM ADP into the cuvette and converting it to ATP, the control mass of mitochondria in the case of V_3 accounted for 25.6 μg atoms O_2/min consume large amounts of oxygen. In the case of V_3, when exposed to dihydroquercetin at concentrations of 20, 60, and 100 μM, oxygen consumption by mitochondria is 27.6 μg, 33.9 μg, and 41.8 μg, respectively. atom was O_2/min. However, in the case of V_4, the controlled mitochondrial oxygen uptake was 4.54 μg formed atoms O_2/min. When exposed to the above concentrations of dihydroquercetin, it is 4.61 μg, 5.23 μg and 6.47 μg atoms was O_2/min. When dinitrophenol (DNF), a mitochondrial oxidative phosphorylation process

is applied out, the substrate is oxidized but not phosphorylated. High concentrations of dihydroquercetin led to an increase in oxygen consumption compared to the control.

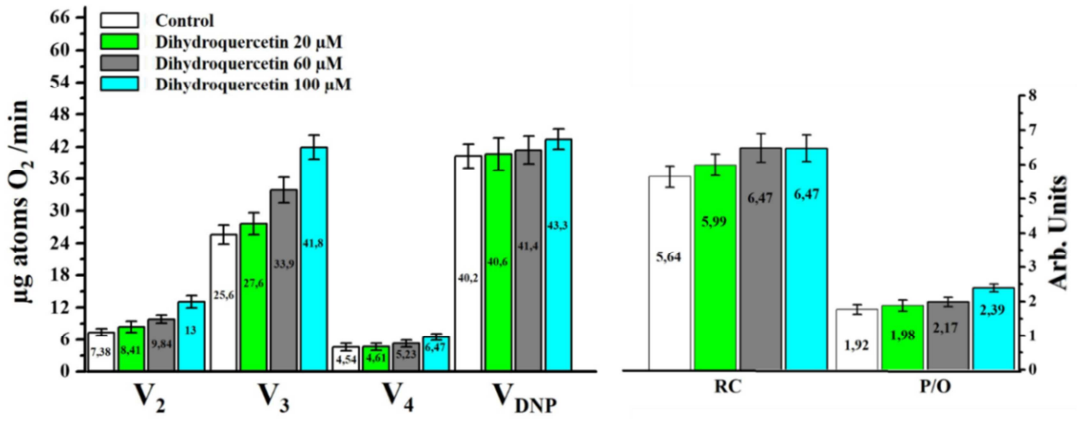

Figure 1. *Effect of dihydroquercetin on the oxidation of FAD-dependent substrates in mitochondria. Effect of dihydroquercetin (O_2 µg/ml) on mitochondrial respiration rate V_2, V_3, V_4, V_{DNP} conditions and respiratory control, ADP/O values. Incubation medium: 100 mM sucrose, 75 mM KCl, 10 mM succinate, 2.5 mM KH_2PO_4, 10 mM Tris-HCl, 5 mM rotenone; pH-7.4. ($P < 0.05$ $P < 0.01$, $n = 5$).*

Respiratory control (V_3/V_4) corresponds to an increase in dihydroquercetin breath control based on both the above indicators and the Chance. An increase in the ADP/O ratio was also observed under the influence of dihydroquercetin (Figure 1). Thus, dihydroquercetin concentrations of 20, 60, 100 µM were significant and partially exceeded the value of ADP/O in respiratory control.

In our subsequent experiments, we investigated the effect of isoquinoline alkaloids F-18 at concentrations of 20, 60, and 100 µM on the oxidative phosphorylation of the FAD-dependent substrate in liver mitochondria. Respiratory oxygen consumption in the V_2 state of liver mitochondria in control rats was 8.06 µg atoms O_2 /min formed. When the mitochondrial metabolic processes of the liver are exposed to the alkaloid F-18 isoquinoline at concentrations of 20, 60 and 100 µM, the oxygen consumption by mitochondria in the V_2 state is 8.51 µg, 10 µg, respectively. and 10.7 µg atoms O_2 /min became overly controlled. Oxidation of the succinate substrate and oxygen consumption by the rate of mitochondrial respiration V_3 under the influence of the isoquinoline alkaloid F-18 (control 25 µg atoms O_2 /min), respectively, 28.6 µg, 33.1 µg and 37.3 µg atoms O_2/min found that the oxygen consumption in the V_4 state of the respiratory rate was controlled by 4.25 µg. atoms O_2 /min. formed. Oxygen consumption was estimated at 4.7 µg and 5.14 µg, respectively, when the incubation medium was exposed to isoquinoline alkaloid F-18 at concentrations of 20, 60, and 100 µM. and 5.59 µg atoms O_2/min. Ha. When DNF (5 µM) was added to the incubation medium, oxygen consumption by mitochondria was controlled at a level of 36.2 µg atoms O_2/ min., isoquinoline alkaloid F-18 at concentrations of 20, 60 and 100 µM, respectively, 37.6 µg, 40 µg and 42.7 µg atoms O_2/min formed. This indicates that under the influence of alkaloids, the oxidation process is somewhat more active than in the control. During the oxidation of the succinate substrate, mitochondrial respiratory control was calculated by Chance. In the control, an increase in the frequency of respiration was noted under the influence of the alkaloid F-18 isoquinoline, according to Chance. It was noted that the ADP/O ratio also increased under the influence of the isoquinoline alkaloid F-18 (Figure 2).

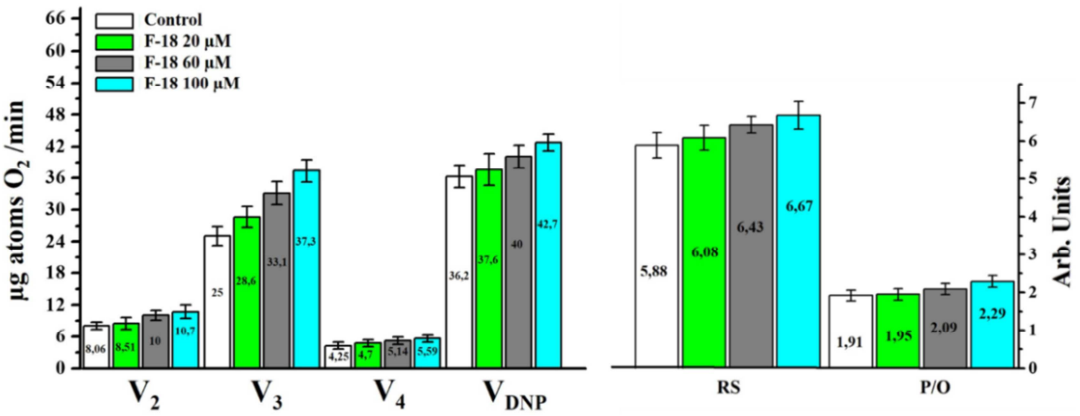

Figure 2. *Effect of isoquinoline alkaloid F-18 on the oxidation of FAD-dependent substrates in mitochondria. Effect of isoquinoline alkaloid in F-18 (O_2 µg/ml) on mitochondrial respiration rate V_2, V_3, V_4, V_{DNP} conditions and respiratory control, ADP/O values. Incubation medium: 100 mM sucrose, 75 mM KCl, 10 mM succinate, 2.5 mM KH_2PO_4, 10 mM Tris-HCl, 5 mM rotenone; pH-7.4. ($P < 0.05$ - $P < 0.01$, $n = 12$).*

In our next experiment, we determined the effect of the DHQ-11 conjugate based on dihydroquercetin and isoquinoline F-18 alkaloids under conditions of substrate oxidation to FAD. Oxygen consumption by mitochondria in the V_2 state is 9.69 µg and 10.8 µg, respectively, at DHQ-11 conjugate concentrations of 20, 60, and 100 µM and 12.2 µg atoms O_2/min found that the oxygen consumption in the case of mitochondrial respiratory rate V_2 obtained as a control was 8.91 µg atoms O_2 /min formed. Oxygen consumption in the V_3 state of the mitochondrial respiration rate was controlled at 28.6 µg atoms O_2/min the turned out to be the mitochondrial respiration rate in V_4 is 4.86 µg atoms O_2/min, DHQ-11 conjugate at concentrations of 20, 60 and 100 µM compared with the control 5.44 µg, 6.02 µg, 6.69 µg the acceleration of the atoms up to O_2/min was found. We found that higher concentrations of DKV-11, when exposed to DNP, accelerated the rate of respiration compared to controls (Figure 3). The concentration-dependent increase in respiratory rate in the case of V_3 and the unchanged change in respiratory rate in the case of V_4 led to a sharp increase in his respiratory control. An increase in the ADP/O ratio was also revealed under the influence of the DHQ-11 conjugate (Figure 3).

The results showed that rats treated with DHQ-11 increased oxygen consumption by activating the respiratory rate in V_3 mitochondria compared to the alkaloids dihydroquercetin and isoquinoline F-18. The effect of the DHQ-11 conjugate on the V_4 respiratory rate was close to that of the alkaloids dihydroquercetin and isoquinoline F-18. The DHQ-11 conjugate enhanced breath control in Chance compared to the isoquinoline alkaloid F-18, but no change was seen with dihydroquercetin. However, DHQ-11 conjugated mitochondria were found to increase the ADP/O ratio more effectively than both compounds. This indicates a slight increase in ATP synthesis in mitochondria in the presence of the DHQ-11 conjugate.

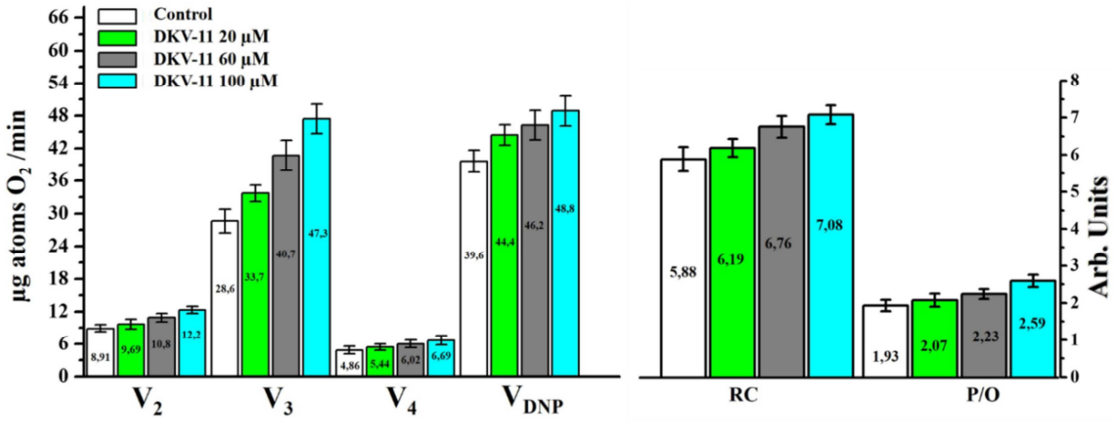

Figure 3. *Effect of DHQ-11 conjugate on the oxidation of FAD-dependent substrates in mitochondria. Effect of DHQ-11 conjugate (O_2 µg/ml) on mitochondrial respiration rate V_2, V_3, V_4, V_{DNP} conditions and respiratory control, ADP/O values. Incubation medium: 100 mM sucrose, 75 mM KCl, 10 mM succinate, 2.5 mM KH_2PO_4, 10 mM Tris-HCl, 5 mM rotenone; pH-7.4. ($P<0.05$ -$P<0.01$, $n = 5$).*

4. Conclusion

Dihydroquercetin concentrations of 100 µM during succinate oxidation in rat liver mitochondria exceeded according to Chance's respiratory rate by 15%. As to the the concentration of isoquinoline alkaloid F-18 100 µM exceeded the control norm by 13%. Concentrations of the DHQ-11 conjugate at a concentration of 100 µM exceeded the respiratory rate by 20% were defined. In mitochondria, the oxidation of FAD-dependent substrates was more actively influenced by the DHQ-11 conjugate than the alkaloids dihydroquercetin and isoquinoline F-18.

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References

[1] Babkin VA, Ostroukhova LA, Ivanova SZ (2004) Products of deep chemical processing of larch biomass. Rus. chem. J. m. XLVIII, 3: 62 (in Russian language).

[2] Baev A. Yu., Angelova P. R., Abramov A. Yu. (2020) Inorganic polyphosphate is produced and hydrolyzed in F_0F_1-ATP synthase in mammalian mitochondria. Biochemical Journal 477: 1515–1524.

[3] Biasutto L, Sassi N, Mattarei A, Marotta E, Cattelan P, Toninello A, et al. Impact of mitochondriotropic quercetin derivatives on mitochondria. Biochim. Biophys. Acta 2010; 1797: 189–196. pmid: 19835835.

[4] Bridges H. R., Jones A. Y., Pollak M. N., Hirst J. Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria // Biochem. J. - 2014. - V. 462. - P. 475-487.

[5] Cao, G.; Sophich, E.; Prior, R. L. (1997) Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Radik Biol Med. 749–760.

[6] Drahota Z., Palenickova E., Endlicher R. et al. Biguanides inhibit complex I, II and IV of rat liver mitochondria and modify their functional properties // Physiol. Res. – 2014. - V. 63 (1). - P. 1-11.

[7] Dzhumaev I. Z., Usmanov P. B., Zhurakulov Sh. N., Vinogradova V. I. (2019) Description of the mechanism of action of alkaloids F-24 and H-14 on the Ca^{2+} transport systems of cardiomyocytes // Infection, Immunity and Pharmacology No. 3 P. 21-27Dzhumaev I., Usmanov P., Rustamov S., Zhurakulov S.(2020) Comparative inotropic effects of some isoquinoline alkaloids. Biomed Pharmacol J. 13 (1).

[8] Hernandez E.-Aquino and P. Muriel, (2018) Benefits of Naringenin in Liver Disease: Molecular Mechanisms, World Journal of Gastroenterology, vol. 24, no. 16, p. 1679–1707.

[9] Kichinska A., Yarmushkevich V. (2020) Flavonoids and mitochondria: activation of cytoprotective pathways? Molecules 3060 8 out of 22.

[10] Peterson G. L. (1977) Simplification of the protein analysis method by Lowry et al. what is more widely applicable // Analytical biochemistry. T. 83 (2). 346-356.

[11] Usmanov P., Dzhumaev I., Rustamov S., Zaripov A., Esimbetov A., Zhurakulov S., Vinogradova V. (2021) Combined inotropic and vasodilating effect of DHA-11, dihydroquercetin flavonoid conjugate with isoquinoline alkaloid 1-aryl-6, 7-dimethoxy-1,2,3,4-tetrahydroisoquinoline. Biomed Pharmacol J; Volume. 14 (2), p. 651-661.

[12] Voyer P. D. The ATP synthase – a splendid molecular machine // Ann. Rev. Biochem, 1997; V. 66: P. 717-749.

[13] Weidmann A. E. (2012) Dihydroquercetin: more than just an impurity? European Journal of Pharmacology. Vol. 684, no. 1-3, p. 19-26.

[14] Zhurakulov Sh. N., Babkin V. A., Chernyak E. I., Morozov S. V., Grigorev I. A., Levkovich M. G. and Vinogradova V. I. (2015) Aminomethylation of 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines by dihydroquercetin. Chemistry of natural compounds. T, 51, №1, 57-61.