Effects of dihydroquercetin, 1-aryltetrahydroisoquinoline, and conjugate on the functional condition mitochondrial membrane of the rat liver

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Abstract

In the current research paper, the flavonoid dihydroquercetin, 1-(2′-bromine-4′,5′-dimethoxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-18) and 2-(3,4-dihydroxyphenyl)-6-(1-(2′-bromine-4′, 5′-dimethoxyphenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2 (IN)-il) methyl-3. The effects of 5,7-trigidroxychroman-4-on (DHQ-11) conjugate on rat liver mitochondrial calcium megachannels and on lipid peroxidation (LPO) induced using Fe2+/citrate were investigated in vitro experiments. White male rats weighing 180-200 grams were used in the experiments. It was found that the DHQ-11 conjugate was identified to have an inhibitory effect on rat liver mitochondria to calcium megachannels and peroxidation of lipids induced by Fe2+/citrate. The inhibitory properties of DHQ-11 conjugate on hepatic mitochondrial calcium megachannels and mitochondrial membrane lipid peroxidation were identified as active against dihydroquercetin and the F-18 isoquinoline alkaloid.

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Introduction

These days, biologically active substances that are being extracted from plants are widely utilised in medicine for the purpose of treatment and prevention of diseases. This is because substances that are derived from plants are distinguished from synthetic drugs by their non-toxic effects on cells and their high biological activity in small concentrations. Cell mitochondria serve as molecular targets for a number of biologically active compounds. Being located in the outer and inner membrane of the mitochondria, the number of ion transport channels modulated by specific activators and inhibitors (Drahota et al. 2009). The calcium megachannel (mitochondrial permeability transition pore-mPTP) can modify its physiological conformation due to increased loading of Ca2+ ions in the matrix (Paul et al. 2008). Currently, there are many tools that could increase mPTP permeabilization. However, pharmaceuticals that inhibit mPTP permeability are encountered quite
rarely (Shengrong et al. 2002). Many flavonoid compounds extracted from plants are considered to physiologically regulate mPTP permeability by inhibiting mitochondrial swelling. One of such biologically active substances, the flavonoid dihydroquercetin, distinguishes itself from other polyphenolic compounds by its biological activity.

Dihydroquercetin is estimated to be the main flavonoid compound isolated from the Larix sibirica tree. Currently, the bioactive properties of the dihydroquercetin flavonoid are being investigated in a number of scientific laboratories around the world. Dihydroquercetin stabilizes cell membranes by inhibiting the formation of free radicals formed as a result of lipid peroxidation (LPO) in biomembranes. It has been found that it inhibits the development of dystrophic and sclerotic changes in tissues as well as normalizes capillary vascular permeability (Babkin et al. 2003). The effects of dihydroquercetin on antioxidant activity in blood cells, platelet aggregation, and erythrocyte hemolysis have been identified (Chen et al. 2009).

Dihydroquercetin has been clinically evaluated and clinical trials have revealed that it has strong natural antioxidant properties (Babkin et al. 2004). Among 1-Aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines, sedative-anxiolytic (Mirzaev et al. 2017), cytotoxic (Terentyeva et al. 2019), antiarrhythmic, anti-inflammatory (Rakhmanova et al. 2022), atypical neuroleptic (Mirzaev et al. 2020) activities, and regulating the volume of thymocytes (Terentyeva et al. 2016) substances have been found within their active characteristics. Some authors have explored the inotropy and antiarrhythmic effects of isoquinoline alkaloids on heart muscle tissue (Zhumaev et al. 2020), as well as the mechanisms of action on cardiomyocyte Ca\(^{2+}\) transport systems (Zhumaev et al. 2019). Taking for experimental purposes 1-(2′-bromine-4′,5′-dimethoxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-18), 3,4-dimetoxyphynilethylamine, and 2-bromine are considered as compounds synthesized on the basis of 4,5-dimethoxybenzaldehyde, which is characterized by its high physiological effect on biomembranes. Among the chemical compounds with heterocyclic structure, isoquinoline alkaloids possess a range of physiological effects. Isoquinoline alkaloids have been investigated for their cardiovascular, antispasmodic, analgesic, and anti-inflammatory quality. The effect of the F-18 isoquinoline alkaloid on papillary muscle contraction in rats has been studied in vitro (Rustamov et al. 2021). Nowadays, intensive scientific research is being conducted on the effects of existing compounds on membrane ultrastructures. Dihydroquercetin flavonoid, 1-(2′-bromine-4′,5′-dimethoxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-18) synthesized on the basis of these compounds 2-(3,4-dihydroxyphenyl)-6-(1-(2′-bromine-4′, 5′-dimethoxy-phenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2 (1N)-il methyl-3,5. The effect of 7-trigidroxychroman-4-on (DHQ-11) conjugate on biomembranes due to structural activity can reduce membrane LPO intensity and effectively affect the functional activity of the cell’s energy organoid mitochondria. However, since the mechanisms of action of dihydroquercetin flavonoid and F-18 isoquinoline alkaloid and their conjugate (DHQ-11) on the liver mitochondrial membrane have not been studied, we aimed to study the effect of these bioactive substances on the LPO process and calcium megachannel in mitochondria.

Experimental

Experimental chemistry part

1H NMR spectrum was registered on UNITY-400 spectrometers (solvent CDCl3, internal standard-HMDS). The Rf values were determined by TLC on silica gel LS 5/40 plates utilizing a chloroform: methanol 6 :1 elution system. The temperature of substance melting point 3 was determined by a ‘BOETIUS’ microtable.

Dihydroquercetin (DHQ), chemical name: 3,5,7,3′,4′-penta-hydroxyflavanonone obtained from Larix sibirica (Babkin et al. 2004) (CAS No. 480-18-2), 1-(2′-bromo-4′,5′-dimethoxyphenyl), 7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (2) was synthesized according to the previously described method (Zhurakulov et al. 2013), the physicochemical characteristics corresponded to the literature data. Synthesis of 2-(3,4-dihydroxyphenyl)-6-(1-(2′-bromo-4′,5′-dimethoxyphenyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2 (1H)-yl methyl-3,5,7-trihydroxy-chroman-4-one (3), DHQ-11.
A solution of dihydroquercetin (1) (0.15 g, 0.49 mmol) in 5 ml of isopropyl alcohol with stirring for 10 min. The reaction mixture was stirred for 30 min at 30 °C, then 0.05 ml (0.54 mmol) of 30 % formalin solution (d = 1.092) was added dropwise. Precipitation began immediately. The reaction mixture was stirred for another 4 h at 30 °C and left at room temperature for 10 h. The progress of the reaction was monitored by TLC. Then the precipitate was filtered off, washed with isopropyl alcohol. The residue was crystallized from ethanol. Yield 0.31 g (89 %), mp. 169-171 °C (from ethyl alcohol), Rf 0.67 (chloroform : methanol, 6:1).

³H NMR spectrum (400 MHz, CDCl 3, δ, m.d., J/Hz): dihydroquercetin fragment: 3.70 (2H, s, NCH₂), 4.33 (1H, d, J = 11.4, H-3), 4.66 (1H, br. signal, H-2), 5.81 (1H, s, H-8), 6.72, 6.78 (each 1H, d, J = 7.7, H-5’, 6’), 6.88 (1H, s, H-2’); 1-(2’-bromo-4’,5’-dimethoxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline fragment: 2.70, 3.03, 3.21 (4H, all, H-3’, 4’), 3.59 (3H, s, 7-OCH₃), 3.64 (3H, s, 6-OCH₃), 3.81 (6H, s, 4’, 5’-OCH₃), 5.13 (1H, s, H-1), 6.10 (1H, s, H-8), 6.56 (1H, s, H-6’), 6.73 (1H, s H-5), 6.99 (1H, s, 3’).

Compound 2-(3,4-dihydroxyphenyl)-6-(1-(2’-bromo-4’,5’-dimethoxyphenyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2 (1H)-yl) methyl-3,5,7-trihydroxychroman-4-one (3) was obtained by the approach according to (Zhurakulov et al. 2015). The structural formulas of alkaloids were drawn by the ChemOffice 2002, ChemDraw Ultra 7.0 software (Fig. 1).

![Fig. 1. Scheme for the preparation of 2-(3,4-dihydroxyphenyl)-6-(1-(2’-bromo-4’,5’-dimethoxyphenyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2 (1H)-yl) methyl-3,5,7-trihydroxychroman-4-one (3).](image)

Animal treatment

In the experiments on white male rats weighing 180 – 220 g, the International Declaration of Helsinki, the Council of International Organizations of Medical Sciences (CIOMS; 1985) and the Institute of Biophysics and Biochemistry at the National University of Uzbekistan named after Mirzo Ulugbek "In Scientific Research was used in accordance with the regulation on the bioethics of the use of laboratory animals" (report dated 22.02.2019). Rats are immobilized under light ether anaesthesia and decapitated under standard laboratory conditions (20 – 24 °C; natural sunlight; 65 % humidity, food, and water available).

Isolation of mitochondria

Mitochondria were isolated from livers by conventional differential centrifugation described by Schneider and Hageboom (1951). Rat liver was homogenized in a medium containing 250 mM sucrose, 10 mM Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl), 1 mM ethylenediamine tetraacetic acid Na₂-salt (EDTA), pH 7.4 centrifuged at 1,500 x g for 7 min (-2 °C, -4 °C). Mitochondria were sedimented by centrifugation of the supernatant at 6,000 x g for 15 min (-25 °C, -4°C). The final mitochondrial pellet was suspended in a small volume of medium containing 250 mM sucrose, 10 mM Tris-HCl, was kept on ice prior to experiments. The mitochondrial protein content was determined by the Lowry method modified by Peterson (1977).

Measurement of mitochondrial swelling

The state of mPTP was assessed by the rate of Ca²⁺-dependent swelling of mitochondria, recording the light scattering of the suspension of mitochondria...
at 540 nm (He and Lemasters 2003). Experimental conditions (mM): sucrose - 200, KH2PO4 - 1, succinate - 5, Ca2+ EGTA buffer – 0.02, Hepes - 20, Tris-HCl - 20, rotenone – 0.002 μg.mL-1, oligomycin - 1 μg.mL-1, pH = 7.2; additives: CaCl2 - 10 μM, CsA - 10 μM. Incubation medium (mM); under these conditions (in the presence of Ca2+ - EGTA buffer), swelling of mitochondria is considered the opening of mPTP.

Lipid peroxidation as measured by Fe2+/citrate

The induction of Fe2+/citrate-dependent swelling of mitochondria was induced by the addition of 50 μM FeSO4 and 2 mM citrate mitochondrial suspension and recorded on a V-5000 spectrophotometer at 540 nm. The incubation medium contained (mM): Sucrose-250, Tris - chloride-10, EDTA-1; pH 7.4. In this case, the amount of mitochondrial protein was 0.5 mg per 1 mL of the incubation medium. The previously established linear correlation relationship between the intensity of lipid peroxidation (LPO) processes induced by the Fe2+/citrate (and Fe2+/ascorbate) system and the rate of high amplitude swelling of mitochondria makes it possible to judge the state of LPO reactions by the intensity of swelling and use this model as a test system for studying the antioxidant properties of various biologically active substances. Despite the fact that the results obtained by the mitochondrial swelling method used by the authors are indirect, the method itself is distinguished by a short analysis time, relative simplicity, and information content sufficient to establish/confirm the desired properties of the tested compound.

Drugs and chemicals

The following chemical reagents were used: EDTA (Sandoz, Switzerland), EGTA, Tris-HCl (Serva, Germany), sucrose, FeSO4, citrate, KH2PO4, succinate, CaCl2 (Chemreaktivsnab, Russia), Hapes, rotenone, oligomycin, and Cyclosporin-A (Selleck, USA). All reagents were p.a. grade.

Data analysis

The results were analysed statistically using Origin Pro 8.6 (Microsoft, USA). The data were evaluated using a parametric Student’s t-test and are expressed as M ± m. The results that were deemed significant are indicated as follows: * P <0.05 and ** P <0.01.

Results

Conducted experiments revealed that the effect of substances on the PTP conduction of rat liver mitochondria was investigated in vitro. The classic inhibitor Cyclosporin-A (CsA) was applied to inhibit mPTP conduction. Ca2+-bound swelling of the liver mitochondria was induced using 30 μM CaCl2. Mitochondrial swelling caused by Ca2+ ions was taken as a 100% control. In subsequent experiments, the conjugate (DHQ-11) synthesized on the basis of mitochondrial swelling of dihydroquercetin flavonoid and isoquinoline alkaloid (F-18) in the mitochondria of rat liver was studied at a semi-maximal IC50 = 0.5 μM concentration of this mPTP classic inhibitor CsA. In the first experiment, the effect of the dihydroquercetin flavonoid on the liver mPTP was studied. According to accumulated results, the mPTP permeability of dihydroquercetin flavonoid at 10, 25, 50, 75, and 100 μM concentrations formed 9.9 ± 3.4 %, 30.2 ± 2.5 %, 56.7 ± 4.6 %, respectively, compared to the control 73.2 ± 3.2 % and 82.2 ± 3.1 % inhibition were determined (Fig. 2).

In the process of exposing dihydroquercetin concentrations of 10, 25, 50, 75, and 100 μM with CsA (IC50 = 0.5 μM) in an incubation medium, mitochondrial swelling was 44.9 ± 4.6 %, 59.2 ± 3.9 %, respectively, compared to the control. Inhibition of 76.1 ± 2.5 %, 83.9 ± 2.6 % and 95.7 ± 4.1 % was observed (Fig. 2). Experimental results indicate that the dihydroquercetin flavonoid inhibited hepatic mPTP permeability, while a pore inhibitor in combination with CsA demonstrated a stronger inhibition of permeability.

In our following experiment, the effect of the F-18 isoquinoline alkaloid on the mPTP permeability of rat liver was studied. Concentrations of the F-18 isoquinoline alkaloid 10, 30, 50, 70, 90, 120 μM in the hepatic mitochondria were 18.2 ± 3.5 %, 38 ± 2.3 %, 59.8 ± 4.04 %, respectively, relative to the control, which inhibited 72.7 ± 2.3 %, 82.3 ± 2.03 % and 88.8 ± 2.2 % of the mPTP (Fig. 3).
The next experiments, the half-maximum inhibitory concentration of CsA and the F-18 isoquinoline alkaloid at concentrations of 10, 30, 50, 70, 90, and 120 μM were affected by 55.2 ± 3.7 %, 68.1 ± 2.1 %, 80.7 ± 3.4 %, 86.1 ± 3.5 %, 89.8 ± 2.6 % and 94.3 ± 2.9 % respectively, when exposed to mPTP conductivity (Fig. 3). More active inhibition was detected at, respectively.

Proceeding the experiment, the incidence of mitochondrial edema was 54.98 ± 4.1 %, 70.8 ± 2.6 %, and 70.8 ± 2.6 %, respectively, when the DHQ-11 conjugate was exposed to concentrations of 10, 20, 30, 40, 50 μM in the incubation medium in accordance with the semi-maximum inhibitory concentration of CsA, 94 ± 4.6 %, 90.9 ± 2.1 %, and 92.8 ± 2.8 % more active inhibition, respectively (Fig. 4). In our next experiment, the effect of mitochondrial membrane lipids of compounds on LPO induced by Fe²⁺/citrate was studied in in vitro conditions. The Fe²⁺/citrate system leads to a sharp increase in the mitochondrial membrane LPO process, which was taken as 100% in the control score. Under the influence of Fe²⁺/citrate, the barrier function of mitochondrial membranes is impaired, and their volume increases relative to control (Almeida et al. 2006). In experiments, it was defined that a concentration of dihydroquercetin 10 μM inhibited mitochondrial LPO process control by 30.0 ± 2.4 % (Fig. 6A). As the concentration of dihydroquercetin in the incubation medium increased, its inhibitory effect on membrane LPO became more pronounced. At the same time, the concentrations of dihydroquercetin 40, 60, and 100 μM LPO were 67.0 ± 5.2 %, respectively, relative to the control,
decreased by 76.0 ± 4.8 % and 90.0 ± 6.6 %, respectively. Experiments have shown that the semi-maximal inhibitory concentration (IC\textsubscript{50}) of dihydroquercetin in the rat liver mitochondrial membrane was 22.2 μM (Fig. 5A).

In our next experiment, 1-(2’-bromine-4’,5’-dimethoxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-18) (10 – 100 μM) rat liver mitochondrial membrane the effect on LPO induced by Fe\textsuperscript{2+}/citrate was studied (Fig. 5B).

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 5.** The effects of dihydroquercetin (A) and F-18 isoquinoline alkaloid (B) on Fe\textsuperscript{2+}/citrate-induced LPO process of rats' liver mitochondria. (*P <0.05; **P <0.01; n = 6).**

The results revealed that concentrations of 10, 20, and 30 μM of the isoquinoline alkaloid (F-18) in the incubation medium were 34.8 ± 3.2 %, respectively, of the control of hepatic mitochondrial LPO; inhibition contributed 56.9 ± 3.6 % and 62.1 ± 4.1 %, respectively. Their inhibitory effect on mitochondrial LPO was more pronounced when the concentrations of the isoquinoline alkaloid in the incubation medium were increased to 40, 50, and 100 μM. At the same time, 40, 50, and 100 μM levels of isoquinoline were 70.2 ± 3.4 %, respectively, of LPO induced by hepatic mitochondria Fe\textsuperscript{2+}/citrate; Decreases were found to be 73.0 ± 4.8 % and 89.1 ± 5.6 %, respectively. Experiments have demonstrated that the semi-maximal inhibitory concentration (IC\textsubscript{50}) of the liver mitochondrial membrane of the F-18 isoquinoline alkaloid was 17.6 μM (Fig. 5B).

Thus, no significant changes in the effect of dihydroquercetin and isoquinoline alkaloids (F-18) on rat liver mitochondrial LPO intensity were seen. However, the fact that the semi-maximal inhibitory concentration of these compounds on LPO induced by Fe\textsuperscript{2+}/citrate in the mitochondrial membrane is slightly lowered at the isoquinoline alkaloid (F-18) indicates that it is slightly more active than dihydroquercetin. Normally, in an LPO system, antioxidants are balanced and work on the feedback principle. Increased activity of antioxidant compounds such as dihydroquercetin flavonoids leads to a decrease in the generation of free radicals, which in turn alters the properties of lipids located in the membrane.

In subsequent experiments, the conjugate (DHQ-11) synthesized on the basis of dihydroquercetin and isoquinoline alkaloid (F-18). Effects on LPO induced by Fe\textsuperscript{2+}/citrate in mitochondrial membranes isolated from rat liver at concentrations of 10–100 μM were performed in in vitro experiments (Fig. 6). The results present that 10 and 20 μM of DHQ-11 conjugate inhibited hepatic mitochondrial membrane LPO by 47.1 ± 4.1 % and 55.9 ± 4.0 %, respectively, relative to control. When the concentration of DHQ-11 conjugate in the incubation medium was enhanced to 30 and 40 μM, it was clear that its LPO intensity decreased by 64.0 ± 4.4 % and 72.0 ± 3.3 %, respectively, relative to control. When the concentration of DHQ-11 conjugate was increased to 100 μM, the
membrane LPO intensity decreased by 88.2 ± 6.7 % relative to control (Fig. 6).

Fig. 6. Effect of DHQ-11 conjugate on rat LPO process in rat liver mitochondria with Fe²⁺/citrate. (*P <0.05; **P <0.01; n = 6).

It was obvious from the experiments that the semi-maximal inhibitory concentration (IC₅₀) of the DHQ-11 conjugate to the mitochondrial membrane of the rat was 14.4 μM. Hence, the DHQ-11 conjugate (10–100 μM) has an inhibitory effect on LPO intensity in rat liver mitochondria. In this case, the inhibitory property of the DHQ-11 conjugate with Fe²⁺/citrate-induced mitochondrial membrane LPO was active against the dihydroquercetin and isoquinoline alkaloids (F-18).

Discussion

An increased load of Ca²⁺ ions in the incubation medium leads to mitochondria swelling. Mitochondrial swelling caused by Ca²⁺ ions indicates that its PTP has led to a highly conductive state. Exceeding the maximum limit of mitochondrial swelling leads to the release of water and water-soluble molecules from its matrix towards the cytosol. The mPTP blocker was reported to inhibit mitochondrial contraction by cyclosporin A (Basso et al. 2008). It has been detected that the flavonoid dihydroquercetin obtained for the experiment, has an activating effect on the mitochondrial ATP-dependent potassium channel (Gayibov et al. 2021). Concentrations of 10–120 μM isoquinoline alkaloid F-18 have been considered to inhibit mitochondrial edema. At the same time, it was established that a high concentration of the isoquinoline alkaloid F-18, 50 – 120 μM is effectively inhibited by the control of mitochondrial edema caused by Ca³⁺ ions. In this condition, the isoquinoline alkaloid F-18 was synthesized on the basis of 3,4-dimethoxypheneethylamine and 2-bromo-4,5-dimethoxybenzaldehyde, the presence of bromine in the structure might enhance its inhibitory properties. In the DHQ-11 conjugate, dihydroquercetin is chemically bonded to the carbon atom in position 6 of ring A, and the isoquinoline alkaloid F-18 is chemically bonded to the nitrogen atom in position 2 of ring B through the CH₂ bridge. Membrane lipid peroxidation increases in the highly permeable state of liver mitochondria. During the experiments, lipid peroxidation was induced with Fe²⁺/citrate. Thus, there were no significant changes were observed in the effect of dihydroquercetin and isoquinoline alkaloids (F-18) on the intensity of LPO in rat liver mitochondria. However, the fact that the half-maximal inhibitory concentration of these compounds against LPO induced by Fe²⁺/citrate in the mitochondrial membrane is lower for the isoquinoline alkaloid (F-18) indicates that it is somewhat more active than dihydroquercetin. Antioxidants in the lipid peroxidation system work according to a balanced feedback principle under normal conditions. The accelerated activity of antioxidant compounds, such as dihydroquercetin flavonoids, leads to a decrease in the formation of free radicals, which, in turn, changes the properties of lipids located in the membrane.

Conclusions

According to the results achieved, it can be summed up that inhibition of hepatic mitochondrial swelling has been determined. Inhibition of the sharp contraction of mitochondria induced by the DHQ-11 conjugate with Fe²⁺/citrate might depend on their antioxidant activity, the number of hydroxyl groups in the structure, and their mutual arrangement. Moreover, the investigated compounds may have high antioxidant and antiradical properties and may not destroy biomembranes. For further clarification of the...
antioxidant properties of the DHQ-11 conjugate, their effect on the activity of antioxidant enzymes is being investigated.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

**References**


