

Effect of Soforaflavonozone and Narcissine Flavonoids on ATP-dependent Potassium Channels of Rat Cardiac Mitochondria in the Ischemia Model

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To cite this article:

Mirzaolimov Elmurod Ismoilovich, Pozilov Mamurjon Komiljonovich, Abduqaxarov Abdugaffor Soyibjon O'g'li, Tursunov Davronbek Rahmonjon o'g'li, Afzalova Sayyora Abdulahadovna, Nishanbaev Sabir Zaripbaevich. Effect of Soforaflavonozone and Narcissine Flavonoids on ATP-dependent Potassium Channels of Rat Cardiac Mitochondria in the Ischemia Model. *American Journal of Biomedical and Life Sciences*. VVol. 10, No. 2, 2022, pp. 45-48. doi: 10.11648/j.ajbls.20221002.17

Received: March 25, 2022; Accepted: April 11, 2022; Published: April 22, 2022

Abstract: In this study, *in vitro* and *in vivo* experiments, it was studied soforaflavonozone (SFL) isolated from *Alhagi canescens* (Regel) B. Keller & Shap (Fabaceae (Leguminosae)), and narcissine isolated from *Crocus sativus* L. belonging to Iridaceae family. It was studied the effect of narcissine isolated from the plant on ATP-dependent potassium channel (mitoK_{ATP}-channel) activity in rat cardiac mitochondria. Animals of experimental group were divided into 4 groups: I control group (healthy), II experimental group (ischemia model), III experiment group (ischemia + narcissin), IV experimental group (ischemia + SFL). In rats with ischemia a 0.1 ml 0.1% solution of 100 mg/kg adrenaline was administered subcutaneously and peritoneally for 3 days in relative to body weight. The rats that underwent the ischemia model were given oral administration of 10 mg/kg of narcissine flavonoid to group III and 10 mg/kg of SFL flavonoid to group IV orally for 7 days. After that, in the experimental animals carried out electrocardiogram. Mitochondria from rat heart tissue were isolated by differential centrifugation. Cardiac mitoK_{ATP}-channel activity in the presence of ATP in an incubation medium was studied at concentrations of 10–50 μM of SFL and narcissine. Concentrations of 50 μM of SFL and narcissus and 30 μM of diazoxide were also found to have an activating effect on the mitochondrial channel of the heart. In the adrenaline-induced ischemia model, it was found that narcissine and SFL flavonoids restored the mitochondrial conduction permeability of the rat heart.

Keywords: Heart, Mitochondria, MitoK_{ATP}-channel, Adrenaline-induced Ischemia, Soforaflavonozone, Narcissine

1. Introduction

About 30-40% of cardiomyocytes' volume is occupied by mitochondria of cardiac muscle cells. The abundance of mitochondria in cardiomyocytes reflects a high level of tissue metabolism. At the present time, the properties, regulation and physiological significance of the ATP-dependent potassium channel (mitoK_{ATP}-channel) located in the plasma

membrane of the cardiac muscle cell and in the mitochondria, as well as the mechanisms of action of biologically active substances are being studied with great interest. The physiological function of the K_{ATP}-channel, located in the plasma and mitochondrial membranes, plays a key role in maintaining the homeostasis of potassium ions in the cytosol and matrix, potential formation, and volume control. Plasma membrane and mitoK_{ATP}-channels have general physiological properties. That is, their modulators (diazoxide, pinacidil,

glibenclamide) have a close effect on K_{ATP} -channel activity [7]. However, the feature of the $mitoK_{ATP}$ -channel is that the active activators and inhibitors show their activity in small concentrations. A common feature of all potassium channels belonging to this family is their inhibition of ATP by the physiological concentration and their selectivity for potassium. The opening of the $mitoK_{ATP}$ -channel protects heart cells in ischemia. Channel activation in cardiomyocytes inhibits apoptosis caused by oxidative stress [5]. The K_{ATP} -channel plays a physiological role in the secretion of glucose-stimulating insulin in pancreatic β -cells [8]. The mechanisms of dysfunction of $mitoK_{ATP}$ -channel are being actively studied in pathophysiological processes, including ischemia. Because ischemia is a very common disease, the supply of oxygen to the heart muscle cells, disruption of the work cycle, and the formation of free radicals in the cells

characterize it. One of the current problems is the study of the dysfunction of the K_{ATP} -channel of the cardiac mitochondria in the conditions of ischemia and the effect of biologically active substances on them.

The aim of the work. The effect of soforaflavonozone and narcissine flavonoids on the ATF-dependent potassium channel of rat heart mitochondria in a healthy and ischemic model was studied *in vitro* and *in vivo*.

2. Materials and Methods

Soforaflavonozone (SFL) (kempferol-3-O-B-D-sophoroside) is isolated from *Crocus sativus* L. plant belonging to the Iridaceae family. Narcissine is isolated from *Alhagi canescens* (Regel) B. Keller & Shap [2, 3] (Figure 1).

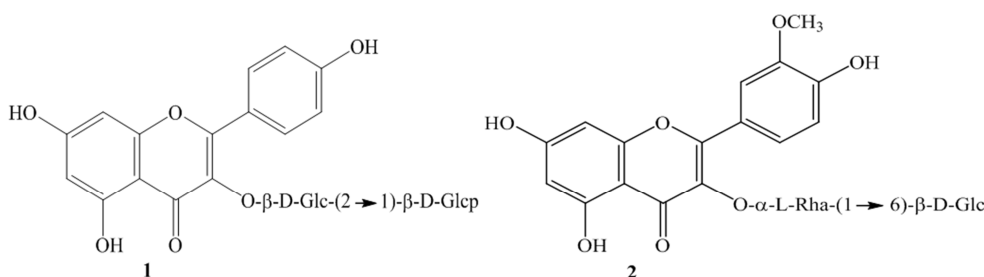


Figure 1. Structural formula of flavonoids Soforaflavonozone (1) and narcissine (2).

2.1. Experimental Animals

The *in vitro* and *in vivo* experiments were performed on male white rats weighing 180-200 g. animals of experimental group were divided into four groups: I control group (healthy), II experimental group (with ischemia model), III experimental group (ischemia + narcissine), IV experimental group (ischemia + SFL). To create a model of ischemia in rats, II, III and IV experimental groups were administered a 0.1 ml of 0.1% solution of 100 mg/kg adrenaline subcutaneously (under the skin of the abdomen) for 3 days in relative to the body weight of the animals. An electrocardiogram was performed to detect pathophysiological changes in cardiac function in rats called *ischemia model*. After it was confirmed that a model of ischemia was formed in the experimental animals, they were given oral administration of narcissine flavonoid to III group 10 mg/kg and IV group orally SFL flavonoid 10 mg/kg for 7 days. After that, in the experimental animals it was performed an electrocardiogram once more. After it was determined that the recovery process was observed on their cardiogram, the mitochondria of the rat cardiac tissue were isolated by differential centrifugation.

2.2. Isolation of Mitochondria from Rat Heart

Mitochondria isolated from rat heart by differential centrifugation according to Schneider [12]. Nuclei and cellular fragments were removed by centrifugation at 600 g for 7 minutes in a centrifuge. The mitochondria are pelleted

at 10000 g for 15 minutes at the same temperature. The mitochondrial pellet was washed twice in the isolation EDTA-free medium.

2.3. Mitochondrial Swelling Measurement

Mitochondrial swelling was estimated from the decrease of absorbance at 520 nm in a V-5000 Visible spectrophotometer at 26°C in a swelling medium of 125 mM KCl, 10 mM HEPES, 5 mM succinate, 1 mM $MgCl_2$, 2,5 mM K_2HPO_4 , 2,5 mM KH_2PO_4 , rotenone 1 μM / ml, oligomycin 1 μM /ml, pH 7,4 [1]. Mitochondria were added to provide the absorbance of about 1 (about 0.4 mg protein per ml).

The mitochondrial protein content was determined by Lowry method with the modification of Peterson [13]. Static analysis of data was performed using the software features Origin 6.1 (Microcal Software Inc., Northampton MA). The P value<0.05 was considered as an indicator of significant differences.

3. Results and Discussion

Until now, many pharmacological agents have been identified that activate or inhibit the $mitoK_{ATP}$ -channel [10]. Diazoxide (used to treat low blood sugar due to a number of specific causes) and nicorandil (used to prevent and treat chest pain caused by angina) acting as activators of the $mitoK_{ATP}$ -channel, and inhibitors include modulators such as 5-ND, MCC-134, and glibenclamide [4]. Similar modulators are very rare and there is increasing attention to their new

types. As a means to identify such modulators, we studied the effect of concentration-dependent SFL and narcissine flavonoids isolated from plants on the $\text{mitoK}_{\text{ATP}}$ -channel of the rat heart *in vitro*.

According to the results, the permeability (control) of the $\text{mitoK}_{\text{ATP}}$ -channel in the absence of ATP in incubation medium was obtained as 100%. In the presence of ATP in the incubation medium, cardiac $\text{mitoK}_{\text{ATP}}$ -channel activity was

inhibited by 62.6%, while SFL activity was found to be significantly unchanged under 10 μM . An increase in the concentration of SFL to 20-30 μM resulted in activation of the cardiac $\text{mitoK}_{\text{ATP}}$ -channel by ATP 17.4% and 21.3%, respectively, relative to the existing conditions (Figure 2, A). SFL at a concentration of 40-50 μM acted on the $\text{mitoK}_{\text{ATP}}$ -channel as an effective activator, but at higher concentrations it was found that the activity is preserved.

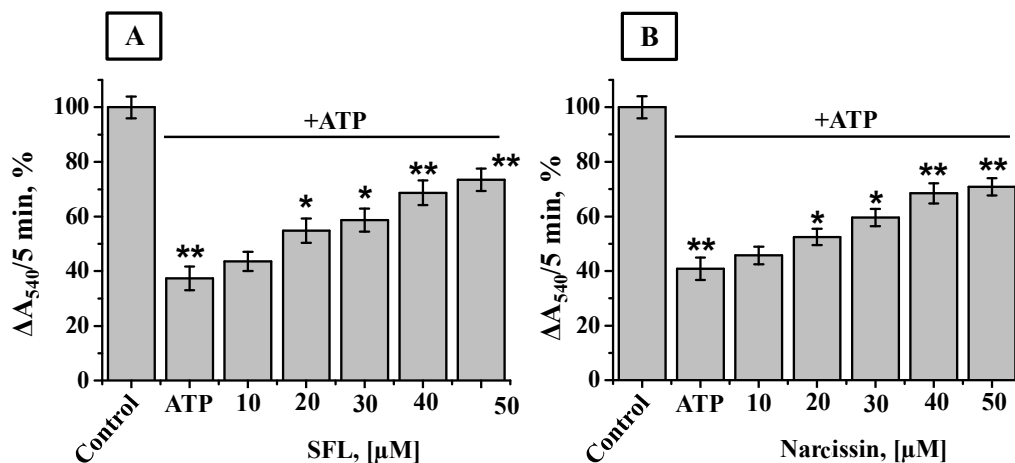


Figure 2. Effect of SFL (A) and narcissine (B) flavonoids on the $\text{mitoK}_{\text{ATP}}$ -channel of the rat heart (* $P < 0.05$; ** $P < 0.01$; $n = 6$).

In our next *in vitro* experiment, the effect of narcissine on cardiac $\text{mitoK}_{\text{ATP}}$ -channel activity was studied. In the ATP presence, cardiac $\text{mitoK}_{\text{ATP}}$ -channel activity was found to be inhibited by 59.2% relative to control (Figure 2, B). High concentrations of narcissine in the incubation medium were found to activate the cardiac $\text{mitoK}_{\text{ATP}}$ -channel relative to the existing conditions of ATP. The effect of narcissine at a concentration of 10 μM was not significant. A concentration of 20 μM of narcissine was noted to slightly activate the activity of the cardiac $\text{mitoK}_{\text{ATP}}$ -channel relative to the existing conditions of ATP. High concentrations of 40 and 50 μM of narcissine were found to increase cardiac $\text{mitoK}_{\text{ATP}}$ -channel activity. Hence, the selected concentrations of 30, 40, and 50 μM of SFL and narcissine had an activating effect on the cardiac $\text{mitoK}_{\text{ATP}}$ -channel (Figure 2, B).

In the next part of the experiment, we studied the activating properties of the classical diazoxide activator at a concentration of 30 μM on $\text{mitoK}_{\text{ATP}}$ channels in the presence of SFL and narcissine 50 μM . At a concentration of 30 μM of diazoxide in the medium, it was found that ATP activated the activity of the $\text{mitoK}_{\text{ATP}}$ -channel by 37.7% compared to the existing conditions. It was shown that the addition of 30 μM diazoxide, 50 μM SFL and narcissine to the incubation medium comprehensively activates the activity of the cardiac $\text{mitoK}_{\text{ATP}}$ channel. In this study, the effect of SFL on cardiac $\text{mitoK}_{\text{ATP}}$ -channel activity was found to be less effective than that of narcissine. Hence, in the presence of diazoxide, SFL and narcissine in the incubation medium, their cardiac mitochondrial activating property was found to increase (Figure 3).

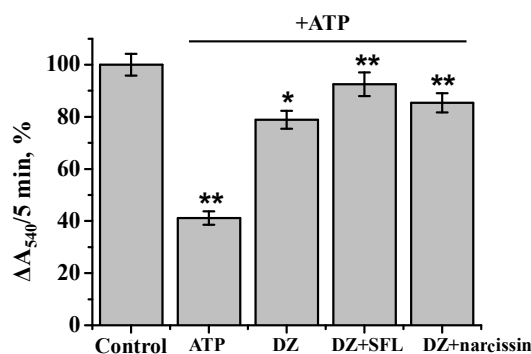


Figure 3. Comparative effect of SFL and narcissine with activator-diazoxide on mitochondrial channel activity in rats (diazoxide-DZ 30 μM , SFL 50 μM , narcissine 50 μM). (* $P < 0.05$; ** $P < 0.01$; $n = 5$).

Up to date, many studies have been conducted on the properties of activators of the cardiomyocytes $\text{mitoK}_{\text{ATP}}$ -channel [6, 7, 10, 11]. However, the effect of SFL and narcissine on the $\text{mitoK}_{\text{ATP}}$ -channel of the rat heart in the ischemic model has not been studied. For this purpose, in our *in vivo* experiments, it was determined the effect of SFL and narcissine on the mitochondrial channel of the rat heart in the model of ischemia. According to the results, when mitochondria were isolated from cardiac tissue in II group rats with ischemia, it was found that $\text{mitoK}_{\text{ATP}}$ -channel activity was inhibited relative to control (I group) (Figure 4).

Rats of III group (with ischemia model) were treated orally with pharmacotherapy for 7 days at a dose of 10 mg/kg of narcissine and mitochondria were isolated from their heart tissue. Cardiac $\text{mitoK}_{\text{ATP}}$ -channel permeability in narcissine -

treated III group of rats was found to be 38.0% more active than in II group of rats. Rats of IV group with ischemia were treated orally at a dose of 10 mg/kg of SFL for 7 days and mitochondria were isolated from their heart tissue. Cardiac mitoK_{ATP}- channel permeability in IV group of rats, treated with SFL was found to be 45.7% more active than in rats of II group.

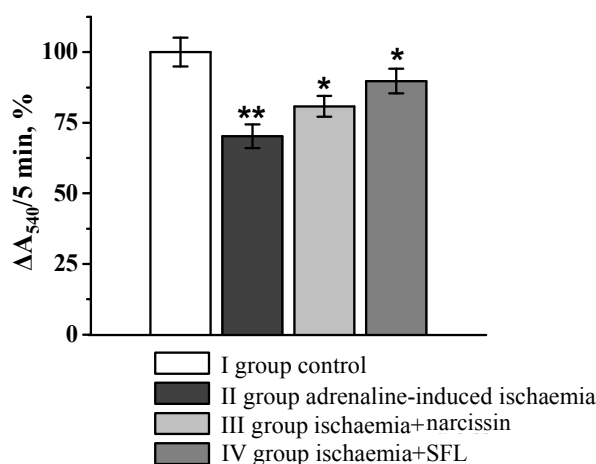


Figure 4. Effect of narcissine and SFL on the mitoK_{ATP}-channel of the rat heart in the adrenaline-induced ischemia model (**P*<0.05; ***P*<0.01; *n*= 6).

It was registered reactivation of mitoK_{ATP}-channels isolated from the heart of rats during the treatment of ischemic animals of III and IV groups during their treatment with SFL and narcissine. In this study, the SFL flavonoid reliably acted as an activator of the mitochondrial channel in rats against narcissine in ischemic conditions (Figure 4). Such substances that activate the K_{ATP}-channel have been shown to depolarize the cardiac mitochondria [11]. However, since the metabolic activators of the mitoK_{ATP}-channel are synthetic analogues, they may cause disruption of the regulation of cellular ion transport systems in relation to natural active substances [6].

4. Conclusions

Flavonoid compounds can control the volume of mitochondria and prevent osmotic suffocation in pathological conditions through the activating and inhibitory effects on the K_{ATP}-channels and mPTP in our experiments with SFL and narcissine [9]. Consequently, SFL and narcissine require further study of their pharmacology as a cardioprotective agent that protect cardiomyocytes from hypoxia and ischemia by activating the cardiac mitoK_{ATP}-channel.

Acknowledgements

We would like to thank the researchers of the Laboratory of Molecular Biophysics of the Institute of Biophysics and Biochemistry at the National University of Uzbekistan for their close assistance in this work.

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