



## MEMBRANE ACTIVE PROPERTIES OF SOME ISOQUINOLINE ALKALOIDS

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### ABSTRACT

In this study, 1- (4-dimethylaminophenyl) -6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-24), 1- (2-chloro-4,5-methylenedioxyphenyl) -2-hydroxyethyl-6, the effect of 7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (N-14) isoquinoline alkaloids on calcium-induced mitochondrial swelling in rat heart mitochondria was studied *in vitro*. Mitochondrial edema was induced using the megachannel inducer  $\text{CaCl}_2$ . The inhibitory effect of different concentrations of isoquinoline alkaloids on mitochondrial edema has been studied. The mPTP permeability of isoquinoline alkaloids has also been studied in comparative experiments with the classic inhibitor cyclophilin D. In experiments, the alkaloids F-24 and N-14 isoquinoline had an inhibitory effect on mitochondrial permeability in rat heart.

**KEYWORDS:** Heart, Mitochondria, Swelling, Isoquinoline alkaloid N-14, F-24.

### INTRODUCTION

Under conditions of calcium overload in the mitochondria, especially with oxidative stress, increased phosphate concentration and decreased adenine nucleotides, a non-specific hole in the inner mitochondrial membrane - mitochondrial permeability transition pore- mPTP) opens [Halestrap A.P. 2009].

The opening of mPTP allows the free passage of proton molecules <1.5 kDa into the mitochondria. The result is the uncoupling of oxidative phosphorylation, decreased ATP synthesis, and necrotic cell death [Šileikytė J. and Forte M. 2019]. One of the main causes of reperfusion injury is now the discovery of mPTPs, the inhibition of which is widely recognized to be an effective goal of cardioprotection [Perrelli M.G. et al., 2011]. Although extensive research has been conducted in many laboratories to determine the physiological properties of mPTP, their molecular identifier remains unclear. Studies have confirmed the role of cyclophilin-D, suggesting that it may contribute to the conformational change of the inner membrane protein due to its peptidyl-prolyl tsis-transisomerase activity [Amanakis G. and Murphy E. 2020].

However, the identification of membrane components remains controversial. Mitochondrial PTP components have been found to be composed of protein compounds, e.g. voltage-dependent anion channel (VDAC), adenine

nucleotide translocase (ANT), mitochondrial phosphate carrier, disordered proteins. However, genetic studies have now ruled out most of these possibilities with  $\text{F}_1\text{F}_0$ -ATPase alone under heated debate [Boyenle I.D. et al., 2022]. Cyclophilin D remains an accepted regulator of mPTP known to date, but no drug has emerged as an inhibitor of it, which raises pressing issues for pharmacologists.

In the development of cardiovascular disease, mPTP plays a key role in the release of cytochrome s from the matrix and the maintenance of membrane potential. PTP acts as a "target" for various pharmacological agents. In doing so, the agents may interact directly with the components of the bribe or by attenuating the effects of mPTP inducers such as the active form of oxygen,  $\text{Ca}^{2+}$  ions, and pH value. Under such conditions, the ATP reserves present inside the cell are rapidly depleted, which in turn disrupts ion and metabolic homeostasis in the cell [Kwong J.Q. and Molкетин J.D. 2015].

The study of modulation mechanisms of cardiac mitochondrial function is important in the determination of cardioprotective agents. Many plant compounds can demonstrate their cardioprotective properties by effectively affecting the mitochondrial ion transport systems of the heart. Among the chemical compounds with a heterocyclic structure, the alkaloids isoquinoline

are considered to have a wide range of physiological effects [Dey P. *et al.*, 2020].

Such compounds include 1-(4-dimethylaminophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-24) and 1-(2-chloro-4,5-methylenedioxyphenyl)-2-hydroxyethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (N-14) isoquinoline alkaloids on  $\text{Ca}^{2+}$ -bound megachannel permeability in rat heart mitochondria has not been studied *in vitro*.

In this study, we aimed to study the effect of concentration of the alkaloids N – 14 and F – 24 isoquinoline on mitochondrial suffocation in rats.

### MATERIALS AND METHODS

Experiments were performed on outbred white rats weighing 180–200 g. Liver mitochondria were isolated by differential centrifugation [Schneider WC, *et al.*, 1948]. The composition of the medium for isolation of liver mitochondria: 250 mM sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4; Mitochondria were resuspended in isolation medium without EDTA.

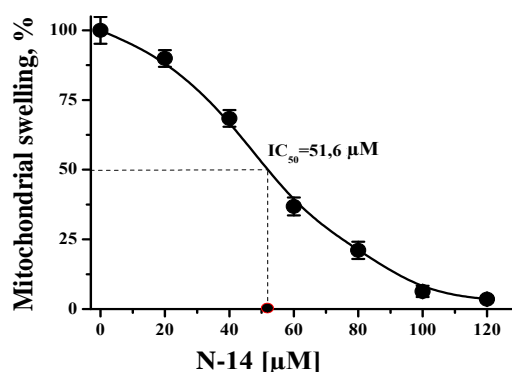
Mitochondrial swelling (0.3–0.4 mg/ml) was determined by changing the optical density of the mitochondrial

suspension in an open cell (volume 3 ml) at 540 nm with constant stirring at +26°C. The following incubation medium was used to determine the permeability of mPTP in mitochondria: 200 mM sucrose, 20  $\mu\text{M}$  EGTA, 5 mM succinate, 2  $\mu\text{M}$  rotenone, 1  $\mu\text{g/ml}$  oligomycin, 20 mM Tris, 20 mM HEPES, and 1 mM  $\text{KH}_2\text{PO}_4$  (pH =7,4).

The content of mitochondrial protein was determined by the method of Lowry modified by Peterson [Peterson GL. 1970]. The Origin 6.1 program was used for statistical processing of the results. A  $P < 0.05$  value was considered as an indicator of significant differences.

### RESULTS AND DISCUSSION

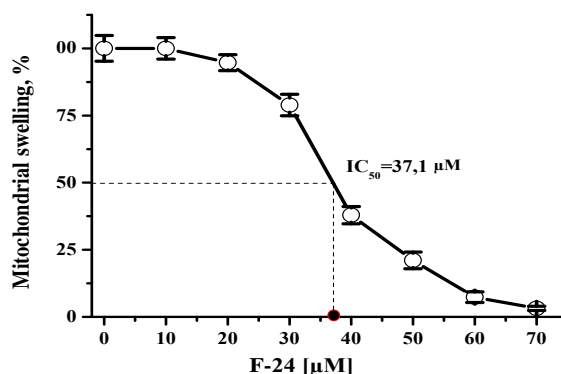
It is known that in the development of cardiovascular disease, mPTP plays a key role in the release of cytochrome *s* from the matrix and the maintenance of membrane potential. The effect of N-14 and F-24 isoquinoline alkaloids on the functional activity of mPTP in rat heart mitochondria was studied *in vitro*. A concentration of 10  $\mu\text{M}$  of  $\text{Ca}^{2+}$  ions was used to induce cardiac mitochondrial edema. The effect of N-14 alkaloid concentrations of 20  $\mu\text{M}$  to 120  $\mu\text{M}$  on the mitochondria of the rat heart was studied (Figure 1).



**Figure 1: Concentration-dependent effect of N-14 alkaloid on mitochondrial swelling in rats.**

Concentration of N-14 alkaloid 20  $\mu\text{M}$  in cardiac mitochondrial swelling (when its suspension was 0.4 mg protein / ml) was inhibited by 10.1% compared to the control value. Cardiac mitochondrial suffocation of the alkaloid N-14 was inhibited by 31.6% at 40  $\mu\text{M}$ , while its maximum inhibitory concentration was 120  $\mu\text{M}$ . The half-maximum inhibitory concentration of the alkaloid N-14 in cardiac mitochondria mPTP was 51.6  $\mu\text{M}$ . Thus, the increase in the concentration of the alkaloid N-14 inhibited the contraction of the mitochondria of the heart due to  $\text{Ca}^{2+}$  ions. This acted as a blocker by inhibiting the conductance of mPTP by the N-14 alkaloid of the cardiac mitochondria.

In subsequent experiments, the effect of alkaloid F-24 on the suffocation of mitochondria in rats was studied at concentrations of 10-70  $\mu\text{M}$ . The results showed that the effect of alkaloid F-24 (10  $\mu\text{M}$ ) on cardiac mitochondrial suffocation was insignificant. A 30  $\mu\text{M}$  concentration of the compound was found to inhibit cardiac mitochondrial mPTP permeability by 21.1%. The maximum concentration of F-24 alkaloid inhibiting cardiac mitochondrial swelling was noted to be 70  $\mu\text{M}$ . It was noted that the semi-maximum inhibitory concentration of the alkaloid F-24 in the mitochondria of the heart due to  $\text{Ca}^{2+}$  ions is 37.1  $\mu\text{M}$  (Figure 2).

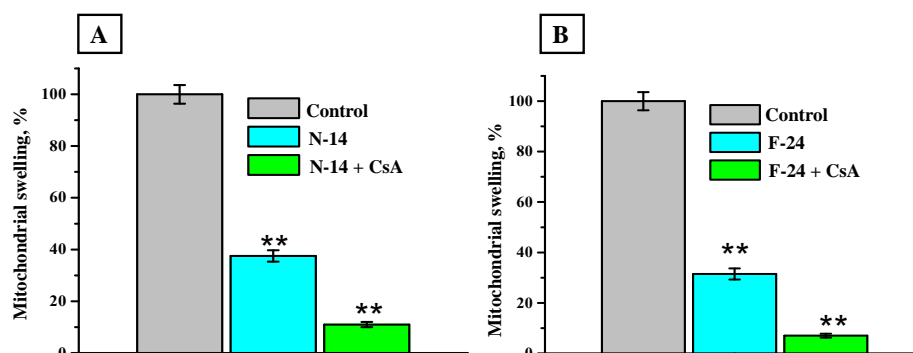


**Figure 1: Concentration-dependent effect of F-24 alkaloid on mitochondrial swelling in rats.**

Thus, the alkaloid F-24 isoquinoline was also found to reduce mitochondrial swelling in rats and partially inhibit mPTP permeability. Under these conditions, mPTP was assumed to be in a closed conformational state. It was hypothesized that inhibition of mitochondrial swelling under the influence of these compounds may be associated with activation of the mPTP component CyP-D or binding of the mPTP complex to the SH-group.

In our next experiment, the effect of mitochondrial swelling in the presence of concentrations of alkaloids

N-14 60 μM and alkaloids F-24 40 μM was studied comparatively with the classic inhibitor of mPTP cyclosporine A (1 μM). The results showed that the concentration of N-14 alkaloid 60 μM inhibited cardiac mitochondrial swelling by 62.5% and F-24 alkaloid by 68.5%. In the presence of N-14 and F-24 isoquinoline alkaloids in an incubation medium, cyclosporine concentrations of 1 μM were found to inhibit cardiac mitochondrial swelling by 89% and 93%, respectively, relative to control (Fig. 3, A and B).



**Figure 3: Complex effect of alkaloids N-14 (60 μM) and F-24 (40 μM) with cyclosporine (1 μM) on mitochondrial swelling in rats.**

Isoquinoline alkaloids N-14 and F-24 can play an important role in protecting muscle tissue from injury by inhibiting the increased permeability of cardiac mitochondria due to  $Ca^{2+}$  and oxidative stress. Occurrence of mitochondrial swelling is associated with megachannel activation in the inner mitochondrial membrane. The isoquinoline alkaloids N-14 and F-24, which inhibit mitochondrial swelling, may typically have antioxidant properties. Therefore, in our future studies, it will be possible to determine their antioxidant activity and describe their cardioprotective effect on cardiac mitochondria.

#### CONCLUSION

In this regard, in the future will be the basis for determining the cardioprotective properties of

biologically active substances and the creation of promising drugs based on them. In addition, cardiomyocyte in cardiovascular disease allows the development of new approaches to the correction of mitochondria with biologically active substances.

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