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Effect of *Polyscias filicifolia* Bailey tincture on $tRNA^{Leu}$ and leucyl-tRNA synthetase activity in isolated pig heart

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The aim of the present study was to investigate the effect of *Polyscias filicifolia* Bailey tincture on the activity of tRNA specific for leucine ($tRNA^{Leu}$) and leucyl-tRNA synthetase under anoxia in isolated pig heart. *Polyscias filicifolia* Bailey tincture did not affect the acceptor activity of $tRNA^{Leu}$ from a control pig heart, but after 20 min of anoxic perfusion with a buffer containing *Polyscias filicifolia* Bailey tincture the activity of organic pyrophosphatase was the same as in control. It has been shown that *Polyscias filicifolia* Bailey protects tRNA molecules from conformational changes.

Polyscias filicifolia Bailey tincture did not change activity of leucyl-tRNA synthetase in norm. After 20 min of anoxic perfusion with a buffer containing *Polyscias filicifolia* Bailey tincture, activity of this enzyme reached the control value, whereas after 90 min of anoxic perfusion with the same buffer activity of leucyl-tRNA synthetase it did not completely restore and reached 77% of the control value. Changes in the activity of leucyl-tRNA synthetase correlated with the changes in the activity of inorganic pyrophosphatase. It has been supposed that *Polyscias filicifolia* Bailey tincture protects the activity of leucyl-tRNA synthetase through the protective action on inorganic pyrophosphatase. Our results have shown that *Polyscias filicifolia* Bailey biomass tincture restores activity of translation machinery components under anoxia.

Key words: anoxia, pig heart, *Polyscias filicifolia Bailey*, tRNA, leucyl-tRNA synthetase

INTRODUCTION

Low oxygen availability (hypoxia) is characterized by inadequate oxygen delivery to the myocardium with a resulting imbalance between oxygen demand and energy supply [1]. All nucleated cells sense oxygen concentration and respond to reduced oxygen availability acutely (within minutes) through the activation of pre-existing proteins and chronically (within hours) through the regulation of gene expression [2]. Oxygen deprivation for 20 to 40 min has caused irreversible histochemical and functional changes in the myocardium [3]. The protein synthesis system is one of the most sensitive to oxygen supply [4, 5]. Under myocardial ischemia and anoxia protein synthesis is altered in the heart [4, 6] and other organs [4, 7]. Changes in protein synthesis under oxygen deprivation may be associated with the reduction of high-energy compounds within cells and disturbances in the translation system [1, 2, 7]. Aminoacyl-tRNA formation catalysed by aminoacyl-tRNA synthetases is a key step in protein synthesis [8, 9]. Activity of tRNA and aminoacyl-tRNA synthetases from pig myocardium changed under total anoxia [10–12].

Various biologically active substances such as adaptogens are able to partially or completely improve disturbances of cell metabolism due to ischemia or anoxia. Plants of the family *Aralaceae*: *Panax ginseng* C.A.Mey, *Eleutherococus senticosus* Maxim, *Aralia manshurica* Rupp. et Maxim, *Polyscias filicifolia* Bailey have characteristics of adaptogens [13–15]. *Panax ginseng* C.A.Mey is the most potent adaptogen in medical practice. It prevents myocardial injury induced by ischemia [16, 17]. Because of the expensiveness of *Panax ginseng*, there are attempts to use other plants of the family *Aralaceae*. One of them is *Polyscias filicifolia* Bailey. *Polyscias filicifolia* Bailey was demonstrated to show a regulatory effect on protein metabolism in rabbit liver during experimental myocardial ischemia [18, 19] and in pig heart under anoxia [20]. However, effects of *Polyscias filicifolia* Bailey on total protein synthesis and on various components of the translation machinery under ischemia and anoxia are poorly understood.

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The aim of the present study was to investigate the effect of *Polyscias filicifolia* Bailey tincture on the activity of the key components of the protein synthesis system – tRNA and aminoacyl-tRNA synthetases under anoxia in isolated pig heart.

MATERIALS AND METHODS

Experiments were done on isolated pig hearts weighing 100–150 g. Pig hearts were obtained from a slaughterhouse after killing pigs. Preparation, control and anoxic perfusion were performed immediately after slaughtering as described in previous articles [10, 12]. Investigating the *Polyscias filicifolia* Bailey action, pig hearts were perfused under normoxic and anoxic conditions with a buffer containing a tincture of *Polyscias filicifolia* Bailey (0.5 ml tinct./1000 ml buffer).

Polyscias filicifolia Bailey biomass was obtained from Dr. V. A Kunakh, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine. *Polyscias filicifolia* Bailey tincture was prepared according to requirements for preparation of tinctures by adding 10 ml 70% ethanol to 1 g *Polyscias filicifolia* Bailey biomass. Extraction was carried out for 7 days in the dark. Then the tincture was poured off and 70% ethanol was added till 10 ml.

Preparations of total tRNA were obtained by phenol deproteinization of pig heart extract and by further chromatography on a DEAE-cellulose column according to Brungraber [21] with the subsequent deacylation as described earlier [22]. Preparations of total aminoacyl-tRNA synthetases were isolated by chromatography of pig heart postribosomal supernatant on DEAE-cellulose as described in [23]. Acceptor activity of tRNA^{Leu} in total tRNA preparations isolated from control and anoxic myocardium was measured and renaturation of in-active conformers of tRNA was performed as described in [11]. Activity of leucyl-tRNA synthetase in preparations of total aminoacyl-tRNA synthetases was measured, by the initial rate of tRNA^{Leu} aminoacylation. The reaction mixture contained in 0.25 ml: 0.1 M Tris-HCl (pH 7.5), 0.01 M $MgCl_2$, 0.01 M KCl, 1.0 mM [^{14}C]-leucine, 10 mM ATP, 200 μg of total tRNA preparations and 100 μg of total aminoacyl-tRNA synthetase preparations. The reaction mixture was incubated at 37 °C for 5 min. Addition of the equal volume of 10% ice-cold trichloracetic acid stopped the reaction; the precipitate was washed on nitrocellulose filters. Radioactivity was mea-sured with a Delta-300 liquid scintillation counter. Activity of inorganic pyrophosphatase was measured colori-metrically as described in [12]. The reliability of the data was estimated by Student's distribution coefficient (t). Changes were statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

To investigate the effect of *Polyscias filicifolia* Bailey tincture on the activities of tRNA^{Leu} and leucyl-tRNA synthetase, we chose 20 min as a short-term (reversible changes) and 90 min as a long-term (irreversible changes) anoxic perfusion. In the control group, pig hearts were perfused under normoxic conditions in adequate time-spans (20 min and 90 min). In our previous report, we demonstrated that acceptor activity of tRNA^{Leu} in total preparation of tRNA under 20 min and 90 min of anoxic perfusion decreased [12]. With the aim to evaluate the effect of *Polyscias filicifolia* Bailey tincture on tRNA^{Leu} activity, this preparation was added to the perfusion buffer.

With the aim to evaluate a possible influence of ethanol on the acceptor activity of tRNA^{Leu} and activity of leucyl–tRNA synthetase, we have studied activity of both components of the translation machinery after adding ethanol into the perfusion buffer. The concentration of ethanol was the same as in the tincture of *Polyscias filicifolia* Bailey. No statistical differences were obtained after perfusing with the buffer containing ethanol under normoxic and anoxic conditions.

Figure 1 shows that *Polyscias filicifolia* Bailey tincture did not affect the acceptor activity of tRNA^{Leu} from normoxic pig heart. However, after 20 min and 90 min of anoxic perfusion with a buffer containing *Polyscias filicifolia* Bailey tincture, the acceptor activity of tRNA^{Leu} increased up to the control value after 20 min and up to 75% of the control value after 90 min of perfusion. Diminution of the acceptor activity of tRNA^{Leu} under anoxia may be associated with the formation of functionally inactive tRNA forms due to alterations in the tertiary structure [11, 12]. These alterations may be related to the decrease of pH in the heart muscle under

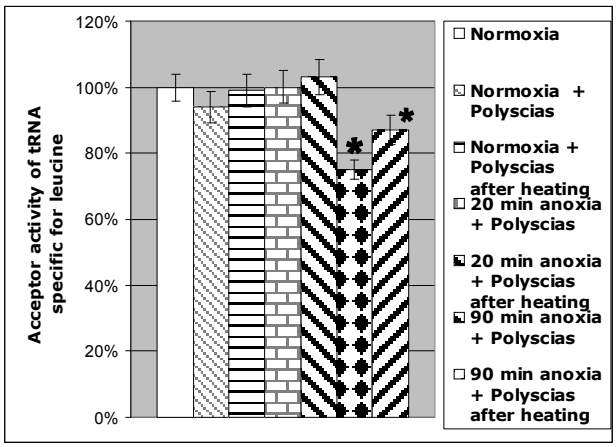

Fig. 1. Effect of *Polyscias filicifolia* Bailey biomass tincture on the acceptor activity of tRNA^{Leu} from control and anoxic pig myocardium and after heating in the presence of magnesium ions

Control (normoxia) represents acceptor activity of tRNA^{Leu} in preparations of total tRNA obtained from pig hearts after perfusion under normoxic conditions with buffer without *Polyscias filicifolia* Bailey tincture.

Data represent results of 8–10 separate experiments.

* Difference between control and experimental group is statistically significant.

anoxia [24, 25]. Magnesium ions are essential to maintain the tertiary structure of tRNA [26]. Short-term treatment (5 min at 60 °C) of tRNA preparations with magnesium ions led to renaturation of tRNA molecules [27].

As reported earlier, treatment of total tRNA preparations with magnesium ions did not affect the acceptor activity of $tRNA^{Leu}$ from normoxic myocardium [11, 12]. However, magnesium ions resulted in a recovery of acceptor activity of $tRNA^{Leu}$ up to the control level after 20 min of anoxia, while in the case of 90 min of anoxic perfusion magnesium ions only partially recovered $tRNA^{Leu}$ activity.

Figure 1 shows also that acceptor activity of $tRNA^{Leu}$ from a normoxic and a 20 min anoxic myocardium, which was perfused with buffer containing *Polyscias filicifolia* Bailey tincture, did not change after treatment with magnesium ions and was as in control. However, treatment of anoxic myocardium after 90 min of anoxic perfusion with a buffer containing *Polyscias filicifolia* Bailey tincture restored the acceptor activity of $tRNA^{Leu}$ from 75% to 87% of the control value. Therefore, it may be assumed that *Polyscias filicifolia* Bailey tincture is a factor that protects tRNA molecules from conformational changes. Our results are in good accordance with the data of other researchers who showed the protective action of *Polyscias filicifolia* Bailey on rabbit liver tRNA when orally administered before experimental ischemia [19].

We studied the effect of *Polyscias filicifolia* Bailey tincture on the activity of leucyl-tRNA synthetase in normoxic and anoxic pig myocardium, because the reliability and level of tRNA aminoacylation depends on the functional activity of aminoacyl-tRNA synthetases [8, 9]. Our previous investigations have shown that different duration of anoxic perfusion has a different effect on leucyl-tRNA-synthetase activity: after 20 min of anoxia it increased, while 90 min of anoxia caused a decrease of this activity as compared to control [12]. Fig. 2 demonstrates that *Polyscias filicifolia* Bailey tincture did not change the activity of leucyl-tRNA synthetase in preparations of total aminoacyl-tRNA synthetases in norm. After 20 min of anoxic perfusion with a buffer containing *Polyscias filicifolia* Bailey tincture, activity of this enzyme reached the control value, whereas after 90 min of anoxic perfusion with the same buffer leucyl--tRNA synthetase activity did not completely restored and reached 77% of the control value. The obtained data confirm reports about the protective action of *Polyscias filicifolia* Bailey on the activity of aminoacyl--tRNA synthetases from rabbit liver under experimental myocardial ishemia [19].

The alterations of aminoacyl-tRNA synthetases activity observed under anoxia may be related to the changes either in the amount or catalytic activity of enzymes. Catalytic activity of aminoacyl-tRNA synthetases is known to depend on various cytoplasmic factors [28]. One of them is inorganic pyrophosphatase which catalyses the cleavage of inorganic pyrophosphate, a potent inhibitor of tRNA aminoacylation.

Earlier we have shown that different duration of anoxia has a similar effect on the activity of inorganic pyrophosphatase and on the activity of aminoacyl-tRNA synthetases; after 20 min of anoxia the activity of inorganic pyrophosphatase increased by 87%, while after 90 min of anoxia decreased by 76% as compared to the control [12]. Data in Fig. 3 reveal the effect of *Polyscias filicifolia* Bailey tincture on the activity of inorganic pyrophosphatase in total preparations of aminoacyl-tRNA synthetases from normoxic and anoxic pig myocardia. *Polyscias filicifolia* Bailey tincture did not

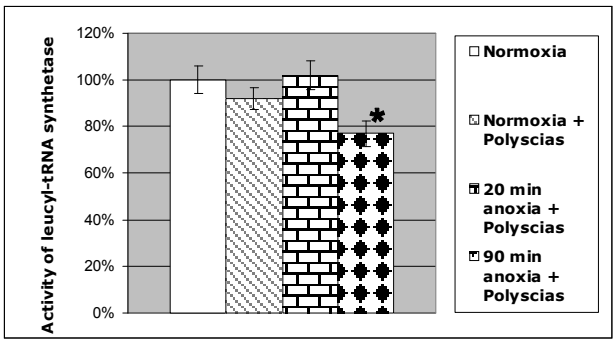

Fig. 2. Effect of *Polyscias filicifolia* Bailey biomass tincture on the activity of leucyl-tRNA synthetase in preparations of total aminoacyl-tRNA synthetases from control and anoxic pig myocardium

Control (normoxia) represents activity of leucyl-tRNA synthetase in preparations of total aminoacyl-tRNA synthetases obtained from pig hearts after perfusion under normoxic conditions with buffer without *Polyscias filicifolia* Bailey tincture.

Data represent results of 8-10 separate experiments.

* Difference between control and experimental group is statistically significant.

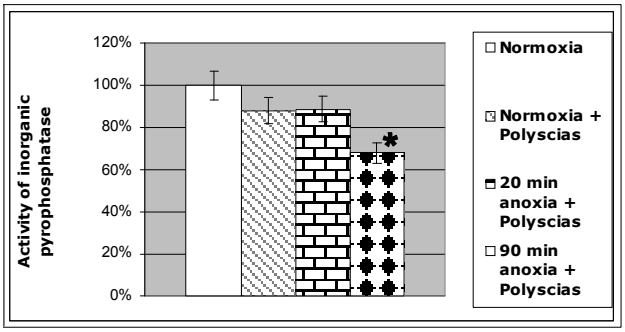

Fig. 3. Effect of *Polyscias filicifolia* Bailey biomass tincture on the activity of inorganic pyrophosphatase in preparations of total aminoacyl-tRNA synthetases from control and anoxic pig myocardium

Control (normoxia) represents activity of inorganic pyrophosphatase in preparations of total aminoacyl-tRNA synthetases obtained from pig hearts after perfusion under normoxic conditions with buffer without *Polyscias filicifolia* Bailey tincture.

Data represent results of 8–10 separate experiments.

* Difference between control and experimental group is statistically significant.

affect the activity of inorganic pyrophosphatase from the control pig heart, but after 20 min of anoxic perfusion with a buffer containing *Polyscias filicifolia* Bailey tincture the activity of inorganic pyrophosphatase increased up to the control value. However, after 90 min of anoxic perfusion with a buffer containing *Polyscias filicifolia* Bailey tincture, the activity of inorganic pyrophosphatase was restored only partially – up to 68% of the control value.

It may be concluded that alterations in the activity of inorganic pyrophosphatase lead to changes in the functional activity of aminoacyl-tRNA synthetases under anoxia. This also suggests that one of the reasons for the restored activity of aminoacyl-tRNA synthetases from anoxic pig myocardium perfused with a buffer containing *Polyscias filicifolia* Bailey tincture was the protective action of the tincture on inorganic pyrophosphatase. Our results have shown that *Polyscias filicifolia* Bailey restores activity of the translation machinery components under anoxia.

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Artūras Kašauskas, Hiliaras Rodovičius, Dalė Vieželienė

POLYSCIAS FILICIFOLIA BAILEY TINKTŪROS POVEIKIS $TRNR^{LEU}$ IR LEUCIL-tRNR-SINTETAZĖS AKTYVUMUI IZOLIUOTOJE KIAULĖS ŠIRDYJE

S a n t r a u k a

Šio darbo tikslas buvo ištirti *Polyscias filicifolia* Bailey tinktūros poveikį leucinui specifinei $tRNR^{Leu}$ ir leucil-tRNR-sintetazės aktyvumui bendruose preparatuose, gautuose iš kiaulių širdžių po perfuzijos, esant anoksinėms sąlygoms. Tiriamoji tinktūra neturėjo įtakos $tRNR^{Leu}$ akceptiniam aktyvumui preparatuose, išskirtuose iš kontrolinės grupės kiaulių širdžių. Perfuzuojant kiaulės širdį anoksinėmis sąlygomis buferiniu tirpalu, kuriame buvo *Polyscias filicifolia* Bailey tinktūros, $tRNR^{Leu}$ akceptinis aktyvumas atsistatė iki kontrolės dydžio po 20 min. anoksijos ir sudarė 75% kontrolės dydžio po 90 min. anoksijos. Manoma, kad *Polyscias filicifolia* Bailey tinktūra apsaugo tRNR molekules nuo konformacijos pokyčių, lemiančių biologinio aktyvumo praradimą.

Polyscias filicifolia Bailey tinktūra neturėjo poveikio leucil-tRNR-sintetazės, katalizuojančios leucino prijungimą prie tRNR, aktyvumui kontrolės grupės preparatuose. Anoksinės perfuzijos sąlygomis, naudojant tinktūrą, leucil-tRNR-sintetazės aktyvumas atsistatė iki kontrolės dydžio esant 20 min. anoksijai, tačiau po 90 min. anoksijos fermento aktyvumas atsistatė ne visiškai ir sudarė 77% kontrolės dydžio. Nustatyta, kad aminoacil-tRNR-sintetazių aktyvumo pokyčiai koreliuoja su neorganinės pirofosfatazės aktyvumo pokyčiais bendruose aminoacil-tRNR-sintetazių preparatuose. Manoma, kad *Polyscias filicifolia* Bailey tinktūra stabilizuoja aminoacil-tRNR-sintetazių aktyvumą, išsaugodama neorganinės pirofosfatazės aktyvumą. Gauti rezultatai rodo, kad *Polyscias filicifolia* Bailey apsaugo transliacijos aparato komponentų aktyvumą anoksijos metu.

Raktažodžiai: anoksija, kiaulės širdis, *Polyscias filicifolia* Bailey, tRNR, leucil-tRNR-sintetazė