ISSN 1607-419X
ISSN 2411-8524 (Online)
УДК 618.2-085

Cardiotonic steroids neutralization as a therapeutic approach in preeclampsia

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Received 24 April 2014; accepted 20 January 2015.

Abstract

Preeclampsia (PE) is a serious complication of late pregnancy. **Objective**. To study and to explore the possibilities of neutralization of cardiotonic steroids, to define the possible mechanisms of recovery activity Na/K-ATPase in pregnant women with PE for the prevention of vasospasm. **Design and methods.** The study was carried out in two stages. Firstly, two groups of pregnant women were included. Control group consisted of 6 women with normal pregnancy with gestational age 37–40–3/7 weeks. The main group consisted of 7 women with PE with a comparable gestational age (mean systolic blood pressure 157 ± 5 mm Hg, diastolic blood pressure 94 ± 2 mm Hg, and urinary protein excretion 2.12 ± 0.46 g/day). At the second stage, 12 patients with PE (aged 29 ± 1 year, gestation 37.9 ± 0.6 weeks, blood pressure 159 ± 5 / 99 ± 3 mm Hg) formed the main group. The control group included 11 healthy pregnant women comparable by gestational age. Venous blood samples were taken. Marinobufagenin level was determined by fluoroimmunoassay, spectrophotometry was used to assess the activity of erythrocyte Na/K-ATPase in the presence and absence of monoclonal antibody, DigiFab, magnesium sulfate. **Results.** The level of marinobufagenin in pregnant women with preeclampsia was almost 2.5-fold higher than in control group (1.056 vs. 0.421 nM). The activity of erythrocyte Na/K-ATPase in pregnant women with preeclampsia was 1.47 ± 0.1716 and 2.65 ± 0.16 mcmol Fn/mL/h ($p < 0.01$), respectively. When incubated with monoclonal antibodies erythrocyte Na/K–ATPase activity was 2.41 mcmol Fn/mL/h. When incubated with DigiFab there was a 3.5-fold increase in PE marinobufagenin plasma levels (1.38 ± 0.40 vs. 0.38 ± 0.10 nmol/L, $p < 0.01$) and a reduction of the erythrocyte Na/K–ATPase activity compared to control group (1.16 ± 0.11 vs. 2.80 ± 0.2 mkmol Fn/ml/h, $p < 0.01$). Ex vivo, 1 ug/ml DigiFab restores the activity of Na/K–ATPase (1.72 ± 0.13 mkmol Fn/ml/h, $p < 0.01$) and 3 mM magnesium sulfate amplifies DigiFab effect (2.3 ± 0.2 mkmol Fn/mL/h, $p < 0.01$).

Key words: preeclampsia, cardiotonic steroids, marinobufagenin monoclonal antibody, DigiFab

For citation: Ishkaraeva VV, Solodovnikova NG, Zazerskaya IE, Frolova EV, Fedorova OV, Bagrov AY. Cardiotonic steroids neutralization as a therapeutic approach in preeclampsia. Arterial'naya Gipertenziya = Arterial Hypertension. 2015;21(1):69–74.

Изучение возможностей нейтрализации кардиотонических стероидов в терапии преэклампсии

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Статья поступила в редакцию 24.04.14 и принята к печати 20.01.15.

Резюме

Преэклампсия (ПЭ) представляет собой тяжелое осложнение второй половины беременности. **Цель исследования** — изучение возможностей нейтрализации кардиотонических стероидов: определение возможных механизмов восстановления активности Na/K-АТФазы у беременных с ПЭ для предупреждения развития ангиоспазма. **Материалы и методы.** Исследование выполнено в 2 этапа. На первом этапе в исследование включены 2 группы беременных. Контрольную группу составили 6 женщин с физиологически протекающей беременностью с гестационным сроком 37–40–3/7 недель. В основную группу вошли 7 женщин с ПЭ с сопоставимым гестационным сроком беременности (среднее систолическое артериальное давление 157 ± 5 мм рт. ст., диастолическое — 94 ± 2 мм рт. ст., экскреция белка с мочой — 2,12 ± 0,46 г/сут). На втором этапе основную группу составили 12 пациентов с ПЭ (в возрасте 29 ± 1 год при сроке беременности 37,9 ± 0,6 недели с артериальным давлением 159 ± 5 / 99 ± 3 мм рт. ст.). Контрольную группу составили 11 здоровых беременных, сопоставимых по сроку гестации и возрасту. У всех беременных производился забор венозной крови. Уровень маринобуфагенина определяли иммунофлуометрическим методом, а активность Na/K-АТФазы эритроцитов спектрофотометрическим методом в присутствии и отсутствии моноклональных антител, DigiFab, сульфата магния. **Результаты.** На первом этапе уровень маринобуфагенина у беременных с ПЭ превысил результат контрольной группы почти в 2,5 раза (1,056 против 0,421 нМ). При этом активность Na/K-АТФазы у беременных с ПЭ составила 1,47 ± 0,1716 против 2,65 ± 0,16 мкмоль Фн/мл/час ($p < 0,01$), при инкубации эритроцитов ПЭ в присутствии моноклональных антител активность Na/K-АТФазы восстанавливается до 2,41 мкмоль Фн/мл/час. При изучении эффектов DigiFab получены следующие результаты. При ПЭ плазменные уровни маринобуфагенина были увеличены в 3,5 раза (1,38 ± 0,40 против 0,38 ± 0,10 нмоль/л, $p < 0,01$), активность Na/K-АТФазы в эритроцитах снижена по сравнению с беременными группы контроля (1,16 ± 0,11 против

2,80 ± 0,2 мкмоль Фн/мл/ч, p < 0,01). Ex vivo при концентрации 1 мкг/мл DigiFab восстанавливает активность Na/K-АТФазы эритроцитов (1,72 ± 0,13 мкмоль Фн/мл/ч, p < 0,01), а 3 ммоль сульфата магния потенцируют эффект DigiFab (2,3 ± 0,2 мкмоль Фн/мл/час, p < 0,01). **Выводы.** Полученные результаты открывают перспективы развития патогенетически обоснованных методов лечения и профилактики ПЭ.

Ключевые слова: преэклампсия, кардиотонические стероиды, маринобуфагенин, моноклональные антитела, DigiFab

Для цитирования: Ишкараева В.В., Солодовникова Н.Г., Зазерская И.Е., Фролова В.Е., Фёдорова О.В., Багров А.Я. Изучение возможностей нейтрализации кардиотонических стероидов в терапии преэклампсии. Артериальная гипертензия. 2015;21(1):69–74.

Introduction

Preeclampsia (PE) is one of the most serious complications of pregnancy in obstetrics. It increases the risk of preterm delivery, fetal development delay, perinatal and maternal mortality [1]. PE causes are currently unclear despite numerous studies [2]. However, endothelial dysfunction, which is accompanied by various factors imbalance, is known to play key role in pathogenesis of the disease.

Recently, the role of cardiotonic steroids (CTS) in hypertension development in PE has been investigated [3–5]. Several endogenous CTS were identified in humans. Ouabain from cardenolides class and marinobufagenin from bufadienolides class are the most studied ones. Marinobufagenin stimulates collagen synthesis in organs of cardiovascular system. In PE, this ketosteroid was found to cause fetoplacental vascular fibrosis leading to the reduction in vascular wall elasticity and its relaxation impairment. The sodium homeostasis and renal natriuresis regulation by linking with Na/K–adenosine triphosphatase (Na/K–ATPase) are physiological functions of endogenous steroids. They both inhibit the transmembrane transport of monovalent cations and activate the Src-EGF-mediated way of cell signaling, causing vasoconstriction [3]. Content of marinobufagenin in blood plasma increases along with the expansion of blood circulating volume and sodium retention in patients with essential hypertension, chronic renal failure and congestive heart failure [6]. After implementation of immunological methods for the individual CTSs determination it was shown that the severe PE development is accompanied by the increase in ouabain and marinobufagenin concentrations [4, 6].

The ways to restore the activity of Na/K–ATPase by neutralizing CTS are under investigation. The potential substances include marinobufagenin monoclonal antibodies. These antibodies were obtained by immunizing of mice with immunogen (marinobufagenin to bovine serum albumin) [7, 8]. DigiFab (BTG International Ltd., UK) — digoxin antibody — is the only one drug approved by Food and Drug Administration. Furthermore, the DigiFab effect with magnesium sulfate (FAB fragments of affinity-purified polyclonal digoxin antibodies) was investigated. Taking into account the therapeutic effect of magnesium sulfate in PE, which might be associated with the ability of magnesium ions to modulate digitalis (CTS) binding with Na/K–ATPase receptor, we hypothesized the probability of synergic interaction of DigiFab and magnesium sulfate in restoring of CTS-inhibited Na/K–ATPase.

The aim of this study was to explore the opportunity to neutralize CTS, namely to determine possible mechanisms of Na/K–ATPase activity recovery in pregnant women with PE for vasospasm prevention.

Design and methods

The study was performed in two stages. Firstly, two groups of pregnant women were enrolled in the study. The control group included 6 women with physiological pregnancy with gestational age 37–40 3/7 weeks without somatic diseases. The main group included 7 women with PE of comparable gestational age of pregnancy. Mean systolic blood pressure in the main group was 157 ± 5 mmHg, diastolic blood pressure — 94 ± 2 mmHg, proteinuria was 2.12 ± 0.46 g/day, the average score by scale according to Savelyeva G.M. — 9 points.

Pregnant women were examined during the first 3–6 hours after admission. Blood sampling in amount of 10 ml in heparinized tubes was performed after physical examination and history taking. From each sample 4 mL of blood were separated by centrifugation (1500 g, 15 minutes). The plasma was frozen for storage at –20 °C and later analyzed by immunofluorometric method in order to assess marinobufagenin by the previously described method [8].

Na/K–ATPase activity was determined in erythrocytes with and without monoclonal marinobufagenin antibodies in 6 ml of blood by previously described procedure [9]. Marinobufagenin antibodies were applied in a concentration, which reverses IC50 to Na/K–ATPase inhibition from medulla renalis in Sprague-Dawley rats (alpha-1 isoform) by the described procedure [10].

Plasma aliquots of patients with PE and of controls were extracted with the use of C-18 reverse-phase cartridge by 80% acetonitrile [11]. The level of CTS in each sample was determined by immunofluorometric method [4].

As the second step, we included two groups of pregnant women in order to investigate the DigiFab effects with magnesium sulfate. The main group consisted of 12 patients with PE aged 29 ± 1 years, with blood pressure of 159 ± 5 / 99 ± 3 mmHg, and gestational age of 37.9 ± 0.6 weeks. The control group consisted of 11 healthy pregnant women aged 30 ± 1 years, with blood pressure averaging 111 ± 2 / 72 ± 2 mmHg and gestational age of 39 ± 0.2 weeks. Blood samples were taken from all the women. We examined the DigiFab effects on Na/K–ATPase activity using spectrophotometric method with and without 3 mmol/l of magnesium sulfate in erythrocytes of patients with PE [9]. The marinobufagenin level was determined by immunofluorometric method [8].

Figure 1. Plasma levels of marinobufagenin in 6 normotensive pregnant women and 7 patients with preeclampsia (p = 0.03)

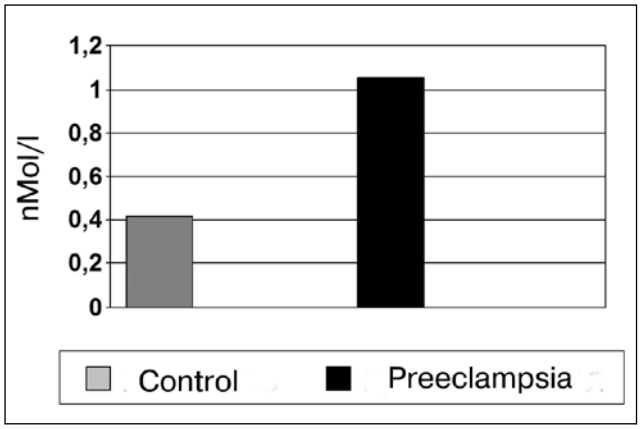
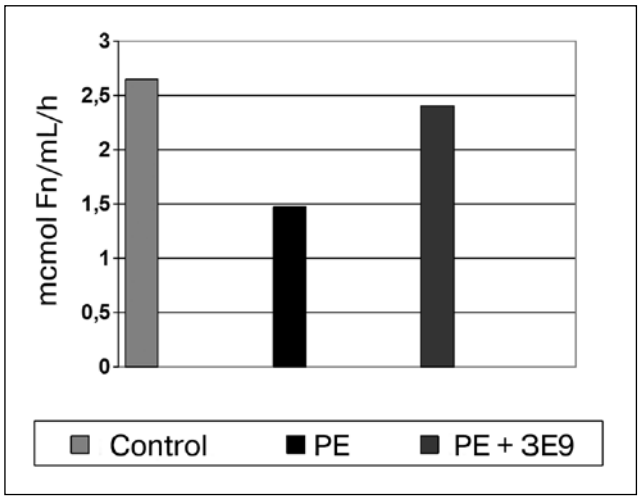


Figure 2. The erythrocyte Na/K–ATPase activity in 6 patients of control group and 7 patients with preeclampsia and erythrocyte incubation with monoclonal antibodies (preeclampsia + 3E9) (p < 0.01)



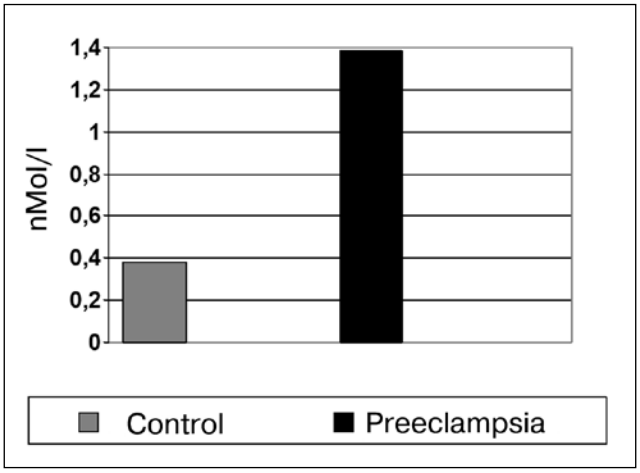
Note: PE — preeclampsia; PE + 3E9 — parameters in pregnant women with preeclampsia during preeclamptic erythrocyte incubation with monoclonal antibodies.

The results were statistically processed (GraphPad Prism 3) using univariate analysis of variance followed by post hoc analysis (Newman-Keuls method). For paired comparison we used t-test.

Results and discussion

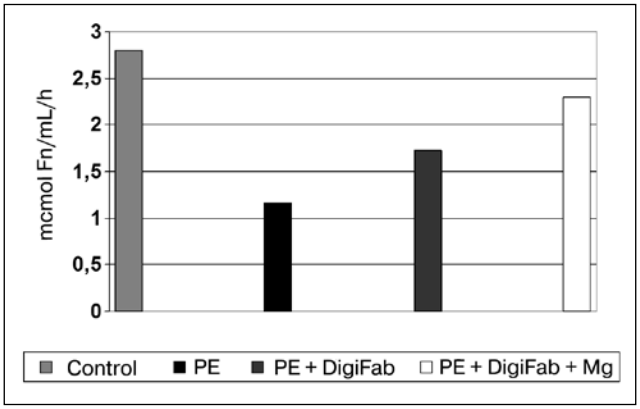
Pregnant women with PE had higher marinobufagenin levels compared to the control group — 1.056 vs. 0.421 nM, respectively (Fig. 1). The activity of Na/K–ATPase was 1.47 ± 0.1716 μmol and 2.65 ± 0.16 μmol/Fn/mL/h in pregnant women with PE and in the control group, respectively (p < 0.01) (Fig. 2). As a result, 2.5-fold increase in plasma marinobufagenin in patients with PE was accompanied by a 50% inhibition of the erythrocyte Na/K–ATPase activity compared to the control group.

Figure 3. Plasma levels of marinobufagenin in 11 normotensive pregnant women and 12 patients with preeclampsia (p = 0.02)



Note: PE — preeclampsia; PE + 3E9 — parameters in pregnant women with preeclampsia during preeclamptic erythrocyte incubation with monoclonal antibodies.

Figure 4. The erythrocyte Na/K–ATFase activity in 11 patients of the control group and 12 patients with preeclampsia in the presence of DigiFab and in combination of DigiFab and magnesium sulphate (p < 0.01)



Note: PE — preeclampsia; DigiFab — digoxin antibody; Mg — magnesium sulphate.

These data confirm the previously reported plasma marinobufagenin increase in PE [5, 8, 11–13]. Marinobufagenin production increase is accompanied by the collagen synthesis enhancement, arteries fibrosis and vasoconstriction development.

The incubation of erythrocytes with monoclonal marinobufagenin antibodies led to reactivating of Na/K–ATPase activity up to 2.41 μmol Fn/mL/h (Fig. 2). Therefore, the addition of antibodies to marinobufagenin in vitro leads to restoration of erythrocyte Na/K–ATPase activity almost to the reference values (90.9%).

In our study, monoclonal antibodies were found to effectively restore the Na/K–ATPase activity in PE by reacting with cardiotonic steroid, particularly, with marinobufagenin.

Monoclonal marinobufagenin antibodies have high activity and selectivity, which gives evidence of their potential use in clinical practice for cardiotonic steroid neutralization in order to treat and prevent vasospasm in PE [12, 14].

The following DigiFab effects were shown. In PE, there was a 3.5 — fold increase in plasma levels of marinobufagenin (1.38 ± 0.40 vs. 0.38 ± 0.10 nmol/L, $p < 0.01$) (Fig. 3), and the erythrocyte Na/K–ATPase activity was reduced compared to control group (1.16 ± 0.11 vs. 2.80 ± 0.2 μmol Fn/mL/h, $p < 0.01$) (Fig. 4). Ex vivo DigiFab 1 μg/ml restores the erythrocyte Na/K–ATPase activity (1.72 ± 0.13 μmol Fn/mL/h, $p < 0.01$), and 3 mMol of magnesium sulfate potentiates the DigiFab effect (2.3 ± 0.2 μmol Fn/mL/h, $p < 0.01$). In vitro magnesium sulphate 3 μmol/l leads to a 12-fold decrease in sensitivity of Na/K–ATPase to the inhibitory effect of marinobufagenin in erythrocytes of the control group.

Our results suggest that cardiotonic steroids neutralization is a component of pathogenetically substantiated treatment of PE. They indicate the potential enhancement of cardiotonic steroid immuneneutralization by magnesium sulfate in PE. The combination of polyclonal marinobufagenin antibodies and magnesium sulfate give new opportunities in PE management and preparing pregnant women for delivery.

Acknowledgements

The study is supported by the Federal Program "Scientific and Scientific-Pedagogical Personnel of the Innovative Russia" (№ 14.740.11.0928).

Conflict of interest

The authors declare no conflict of interest.

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