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COMPARATIVE STUDY OF THE INFLUENCE OF TADALAFIL AND DOXAZOSIN ON THE RATE OF OXYGEN CONSUMPTION BY PROSTATE TISSUE IN SPRAGUE-DAWLEY RATS AND IN WHITE OUTBRED RATS. POSSIBLE CLINICAL CONSEQUENCES OF UNSTUDIED PHARMACOLOGICAL EFFECT IN MEN WITH LOWER URINARY TRACT SYMPTOMS

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Abstract

Objective: to study comparatively the influence of tadalafil and doxazosin on the rate of oxygen consumption (ROC) by prostate tissue in prague-Dawley rats (SDR) and in white outbred rats (WOR).

Methods: 30 SDR and 50 WOR were used. 30 SDR were randomized into 3 groups. 10 SDR (1st group) under general anesthesia (GA) received doxazosin 0.25 mg intragastrically. 10 SDR (2nd group) under GA received tadalafil 0.22 mg intragastrically. 10 SDR (3d group – SDR-control) under GA no drugs received. 50 WOR were randomized into 3 groups also. 17 WOR (4th group) under GA received doxazosin 0.25 mg intragastrically. 17 WOR (5th group) under GA received tadalafil 0.22 mg intragastrically. 16 WOR (6th group – WOR-control) under GA no drugs received. 30 minutes after drug receiving, the prostatectomy was performed. 0.5 grams of prostate tissue was homogenized and put into the glass beaker, containing 15 ml of 0.9% sodium chloride solution at 36.6°C. Then the optic sensor for measuring the concentration of dissolved oxygen (CDO_2) was inserted into the beaker and the tightness was created. The CDO_2 was recorded every minute for 60 minutes. Kruskal-Wallis and Dunn tests were used.

Results: The ROC was higher in the 1st group than in the 3rd group ($p < 0.01$) and in the 4th group than in the 6th group ($p < 0.01$). The ROC was higher in the 2nd group than in the 3d group ($p < 0.05$) and in the 5th group than in the 6th group ($p < 0.05$).

Conclusions: Doxazosin and tadalafil increase the ROC by prostate tissue in both SDR and WOR.

Keywords: *oxygen, pharmacotherapy, doxazosin, tadalafil, the rate of oxygen consumption*

Introduction

"Lower urinary tract symptoms" (LUTS) is the term used to denote typical complaints, associated with voiding, in patients with benign prostatic hyperplasia (BPH), chronic prostatitis (CP) and urinary dysfunction. They include urgency, frequency, incontinence, nocturia, slow stream, and the sensation of incomplete bladder emptying. Analysis of the literature on LUTS gives an opportunity to conclude that ischemic processes in the pelvic organs are one of the main reasons of LUTS [1]. Moreover, there is evidence that convincingly indicates the important or even the main role of ischemia in etiology and pathogenesis of BPH and CP [2].

In 2016, we were the first to detect that the α_1-adrenoblocker doxazosin increases the rate of oxygen consumption by the tissue of prostate in white outbred rats (WOR) [3].

An amazing fact that deserves further comprehensive study, we consider the ability of tadalafil to increase the rate of oxygen consumption by prostate tissue in WOR [4, 5].

However, for the possibility of extrapolating these findings to clinical practice, it is necessary to expand the list of experimental objects. For this, it is necessary to use purebred rats. Another condition is to conduct experiments on expert-grade equipment.

Objective: to study comparatively the influence of tadalafil and doxazosin on the rate of oxygen consumption (ROC) by prostate tissue in Sprague-Dawley rats (SDR) and in white outbred rats (WOR).

Methods

The protocol of this study was approved by the Local ethics committee of the Kazan Medical Academy in 2015.

The rate of oxygen consumption (ROC) by the tissue of prostate was measured using an optical sensor of dissolved oxygen with thermoelectric transformator ДКТП-03 (DKTP-03) (manufacturer – "Econix", Russia). A source of light, a photodetector, and replaceable membrane with a dye are located in sensor housing. The membrane is in the direct contact with the aqueous solution in which the concentration of dissolved molecular oxygen (CDO_2) is measured [6]. This sensor is connected to the analyzer of CDO_2 "Expert-009" (manufacturer – "Econix", Russia) [7].

The sensitive element of the sensor is a special phosphorescent dye. Under the influence of oxygen, the phosphorescence decreases. Based on this, the analyzer calculates CDO_2 [6].

From the physico-chemical point of view, the sequence of stages for measuring oxygen in the aqueous solution is as follows:

1. The sensor's external membrane containing a phosphorescent dye is in direct contact with a 0.9% solution of sodium chloride, in which the CDO_2 is measured.
2. A source of the light, located in the sensor itself, generates the light. This light excites the indicator dye molecules in the membrane.
3. Then there is a transition of molecules from an excited state to an unexcited, or initial, state in one of two ways:

- in the absence of oxygen, energy goes in the form of phosphorescence (this phosphorescence is captured by the receiver inside the sensor);
- in the presence of oxygen, energy is transferred to the oxygen molecules (it is the quenching of phosphorescence) [6, 7].

The higher is the oxygen concentration, the faster is the quenching of phosphorescence. The Stern-Volmer equation describes this relationship. With its help, the analyzer calculates the CDO_2 [6, 7].

This optic sensor of CDO_2 was inserted into the laboratory beaker so that the beaker was completely filled with 15 ml of 0.9% sodium chloride solution at a temperature of 36.6 °C and contained 0.5 g of homogenized prostate tissue immediately after prostatectomy. The tightness was created.

With such contents this beaker was placed into a thermostat at a temperature of 36.6 °C.

The CDO_2 in the 0.9% sodium chloride solution in this beaker was measured for 60 minutes.

30 male Sprague-Dawley rats (SDR) and 50 white outbred rats (WOR) were used. The animals were 6 months old.

30 SDR were randomized into 3 groups: 10 SDR (1^{st} group) under general anesthesia (GA) received doxazosin 0.25 mg intragastrically. 10 SDR (2^{nd} group) under GA received tadalafil 0.22 mg intragastrically. 10 SDR (3^{d} group – SDR-control) under GA no drugs received.

50 WOR were randomized into 3 groups also. 17 WOR (4th group) under GA received doxazosin 0.25 mg intragastrically. 17 WOR (5th group) under GA received tadalafil 0.22 mg intragastrically. 16 WOR (6th group – WOR-control) under GA no drugs received.

We injected 0.5 ml of solution of xylazine 20 mg/ml ("Xyla") intramuscularly into each animal for premedication.

5 minutes after premedication, each animal was injected intramuscularly with "Zoletil-100" in a volume of 0.2 ml. "Zoletil-100" is a mixture of tiletamine hydrochloride and zolazepam hydrochloride in a ratio 1:1. Under anesthesia, the prostatectomy was performed gently. Immediately, the prostate was weighted and homogenized.

Then 0.5 g of prostate tissue was placed into a beaker. The beaker was filled with 15 ml of 0.9% sodium chloride solution at a temperature of 36.6 °C. Then the optic sensor for measuring the CDO_2, connected with the analyzer "Expert-009", was inserted into the beaker. And the tightness was created.

Analyzer calculated CDO_2 values in milligrams per Liter (mg/L). CDO_2 values were recorded every minute for 60 minutes from the start of experiments (0 minute) to the finish of experiments (60 minutes).

In each CDO_2 value, salinity was corrected. For this, a correction coefficient $\alpha = 0.9595$ was used, by which the value of CDO_2 was multiplied [6].

Then the minute CDO_2 values, obtained in all experiments, were placed in one table. In each group (first, second, third, fourth, fifth, sixth) the minute arithmetic means of CDO_2 were calculated. Then the natural logarithm (ln) was calculated for each of them. According to the values of the natural logarithms, the graphics were constructed in the coordinate system "time – ln of CDO_2".

The tangent of the tilt angle of the curve in the coordinate system "time – ln of concentration" is known to be equal to the constant of elimination [8]. In our case, it corresponds to the rate of oxygen consumption by the tissue of prostate.

To check for normality at the start (0 minute) and at the finish of experiments (60 minutes), the D'Agostino Skewness criterion was used in each of the six groups [9].

Then the homogeneities of the first, second and third groups at the start and at the finish of experiments were checked using the Kruscal-Wallis test [10]:

$$H = \frac{n_1 (\bar{R}_1 - R)^2 + n_2 (\bar{R}_2 - R)^2 + n_3 (\bar{R}_3 - R)^2}{N (N+1)/ 12},$$

where $\overline{R_1}$ is the average rank in the group of doxazosin, $\overline{R_2}$ is the average rank in the group of tadalafil, $\overline{R_3}$ is the average rank in the control group, $\mathbf{n_1}$ is the volume of the group of doxazosin, $\mathbf{n_2}$ is the volume of the group of tadalafil, $\mathbf{n_3}$ is the volume of the control group, **R** is the average rank in the total group of all compared objects, **N** is the total volume of all compared groups [10].

Also, the homogeneities of the fourth, fifth and sixth groups at the start and at the finish of experiments were checked using the Kruscal-Wallis test [10].

Results

SPRAGUE-DAWLEY RATS

The results obtained on SDR are shown in Fig. 1.

See Figure 1.

At the start of the experiments, CDO_2 in the SDR-control group was 7.63 ± 0.20 mg/L; in the group of doxazosin it was 7.62 ± 0.16 mg/L; in the group of tadalafil it was 7.63 ± 0.18 mg/L. The homogeneity of the first (SDR-doxazosin), second (SDR-tadalafil) and third (SDR-control) groups at the start of experiments was revealed using the non-parametric Kruscal-Wallis test (the obtained H = 0.07 was less than the critical value = 5.9910, so, $p > 0.05$) [10].

At the finish of the experiments, the following results were obtained. CDO_2 in the SDR-control group was 6.25 ± 0.14 mg/L; in the group of doxazosin it was 3.51 ± 0.27 mg/L; in the group of tadalafil it was 4.34 ± 0.38 mg/L. Statistical differences were checked using the Kruscal-Wallis test. The obtained H = 16.24 exceeded the critical H value = 13.816, so, significant statistical differences between the groups at the finish were identified ($p < 0.001$) [10].

Then the non-parametric Dunn test was used according to the formula:

$$Q = \frac{\overline{R_A} - \overline{R_B}}{\sqrt{\frac{N (N+1)}{12}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)}},$$

where $\overline{R_A}$ and $\overline{R_B}$ are the average ranks of the two compared samples, $\mathbf{n_A}$ and $\mathbf{n_B}$ are their volumes,

and **N** is the total volume of all compared samples [10].

The difference between the SDR-control group and the group of doxazosin was statistically significant (the obtained Q = 3.9 exceeded the critical Q = 2.936; p < 0.01):

$$Q = \frac{24.3 - 8.9}{\sqrt{\frac{30\,(30+1)}{12}\left(\frac{1}{10}+\frac{1}{10}\right)}} = 3.9.$$

The difference between the SDR-control group and the group of tadalafil was statistically significant also (the obtained Q = 2.8 exceeded the critical Q = 2.394; p < 0.05):

$$Q = \frac{24.3 - 13.3}{\sqrt{\frac{30\,(30+1)}{12}\left(\frac{1}{10}+\frac{1}{10}\right)}} = 2.8.$$

The difference between the group of doxazosin and the group of tadalafil was not significant (the obtained Q = 1.12 was less than the critical Q = 2.394; p > 0.05) [10].

WHITE OUTBRED RATS

The results obtained on WOR are shown in Fig. 2.

See Figure 2.

At the start of the experiments, CDO_2 in the WOR-control group was 7.74 ± 0.15 mg/L; in the group of doxazosin it was 7.58 ± 0.11 mg/L; in the group of tadalafil it was 7.71 ± 0.10 mg/L. The homogeneity of the fourth (WOR-doxazosin), fifth (WOR-tadalafil) and sixth (WOR-control) groups at the start of the experiments was revealed using the Kruscal-Wallis test (the obtained H = 0.655 was less than the critical value = 5.991, p > 0.05) [10].

At the finish of the experiments, the following results were obtained. CDO_2 in the WOR-control group was 6.40 ± 0.14 mg/L. In the group of doxazosin it was 3.35 ± 0.15 mg/L. In the group of tadalafil it was 4.39 ± 0.17 mg/L. Statistical differences were checked using the Kruscal-Wallis test. The obtained H = 37.09 exceeded the critical H value = 13.816, so, significant statistical differences between the groups at the finish were identified (p < 0.001) [10].

Then the Dunn test was used. The difference between the WOR-control group and the group of doxazosin was statistically significant (the obtained Q = 6 exceeded the critical Q = 2.936; p < 0.01) [10]:

$$Q = \frac{42.5 - 11.85}{\sqrt{\frac{50\,(50+1)}{12}\left(\frac{1}{16}+\frac{1}{17}\right)}} = 6.$$

The difference between the WOR-control group and the group of tadalafil was statistically significant, too (the obtained Q = 3.8 exceeded the critical Q = 2.936; p < 0.01):

$$Q = \frac{42.5 - 23.18}{\sqrt{\frac{50\,(50+1)}{12}\left(\frac{1}{16}+\frac{1}{17}\right)}} = 3.8.$$

There was no difference between the group of doxazosin and the group of tadalafil (the obtained Q = 2.266 was less than critical Q = 2.394; p > 0.05).

Conclusions

Doxazosin increases the rate of oxygen consumption (ROC) by the tissue of prostate in both Sprague-Dawley rats (SDR) and white outbred rats (WOR). Tadalafil also increases the ROC by the tissue of prostate in both SDR and WOR.

Discussion

Text It is difficult to consider that this pharmacological effect of doxazosin is expected and "logical". The adrenergic system is known to mediate stress reactions. Doxazosin is the α_1-adrenoblocker. Therefore, it would be more logical to expect that it will reduce the ROC. Therefore, we find it interesting that doxazosin *increases* the ROC. Moreover, we did not find such studies in the available literature. In our opinion, this phenomenon may indicate that doxazosin has one more else mechanism of action on prostate tissue, which is realized due to biochemical processes not yet known. This possible, unknown mechanism of action and the classic α_1-blocking effect on the prostate may coexist. This may explain why doxazosin acts individually in patients with benign prostatic hyperplasia (BPH) and chronic prostatitis (CP) [5].

Tadalafil, a phosphodiesterase 5 type inhibitor, has been successfully used in clinical practice since 2002 to treat men with erectile dysfunction (ED) [11]. The results of clinical studies showed the ability of tadalafil to improve urination, to reduce the feeling of incomplete emptying of the bladder and to attenuate the pain in the perineum in men with CP and BPH. This became the reason for administration of tadalafil in men with CP and BPH at a dose of 5 mg orally 1 time per day [12].

However, the mechanism of action of tadalafil on the prostate is still unclear, because the phosphodiesterase 5 type is not contained in the prostate (unlike the cavernous bodies of the penis, where it predominates). Debates have been going on over this for several years [13].

From our point of view, this pharmacological effect studied by us is of great clinical significance. It complements the existing understanding of the effects of doxazosin and tadalafil on the prostate.

First of all, it is possible to understand why tadalafil is effective in men with LUTS associated with CP and BPH. In addition to the fact that doxazosin selectively blocks α_1-adrenoreceptors, and tadalafil inhibits phosphodiesterase 5 type, each of them helps (or makes?) prostate tissue to consume more oxygen.

In addition, this fact may explain why the efficacy of both doxazosin and tadalafil in each patient with BPH and CP is individual: not every patient's vascular system of the pelvic organs can provide prostatic tissue with oxygen, corresponding to the increasing needs of the prostate when taking these drugs. For example, our own experience shows that the clinical efficacy of tadalafil and doxazosin is noticeably less in smokers, in patients with postinfarction cardiosclerosis and atherosclerosis.

Adequate oxygen supply of prostatic tissue, corresponding to the increasing ability of the prostate to consume oxygen when taking doxazosin and tadalafil, may be the condition for achieving the most effective treatment for patients. On the contrary, the inability of the oxygen-transport system to meet the increased oxygen demand of the prostatic tissue when taking doxazosin and tadalafil may cause incomplete clinical efficacy in treating patients with BPH and CP. If oxygen transport is not disturbed, then everything is "in order", and the clinical efficacy of both doxazosin and tadalafil in patients with LUTS will be maximal. And if not (there are many reasons for this), then taking these drugs will be low effective or even uneffective.

So, obtained results give an opportunity to combine the theory of oxygen supply of the organism, the problems of personalized pharmacotherapy in urology, the aspects of biomedical differences between patients (different degree of pelvic ischemia) as the reasons why the clinical efficacy of doxazosin and tadalafil is individual. This subject requires further comprehensive study, because it is very important for the clinical practice.

Disclosure

The authors declare no conflicts of interest.

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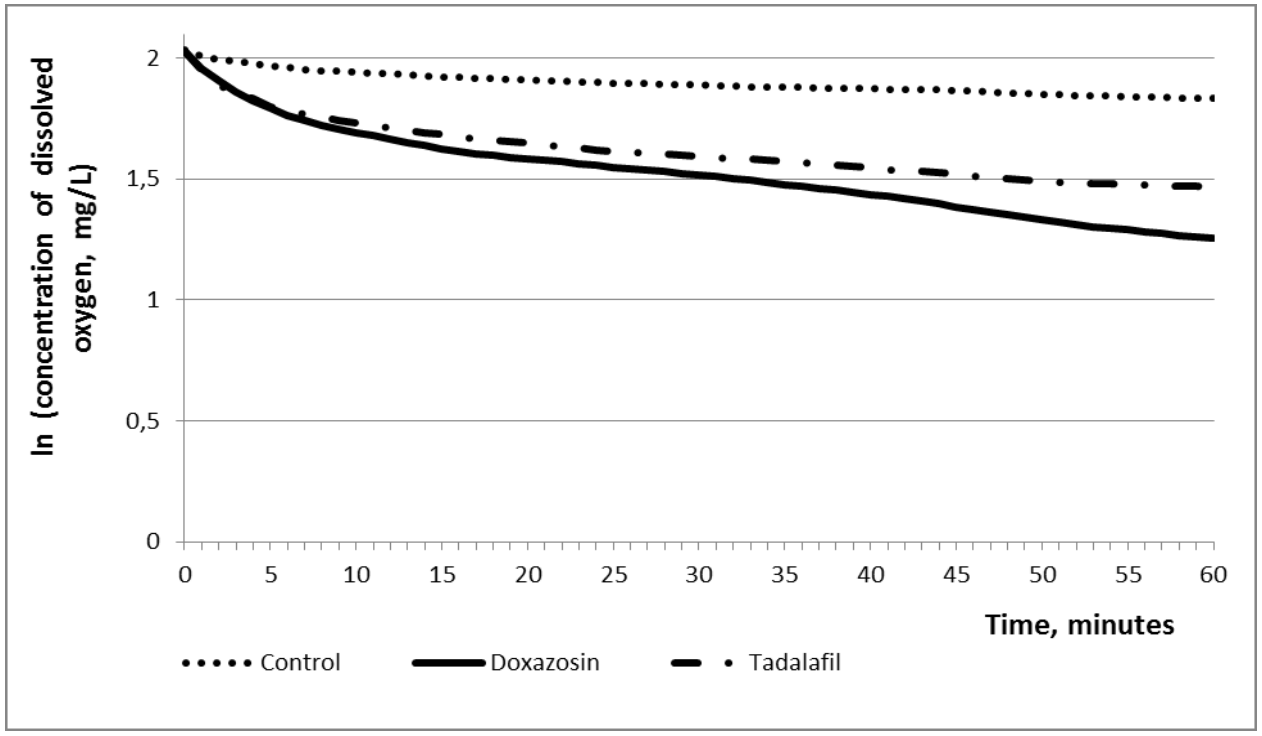

Figure 1. Doxazosin and tadalafil increase the rate of oxygen consumption by the tissue of prostate in Sprague-Dawley rats.

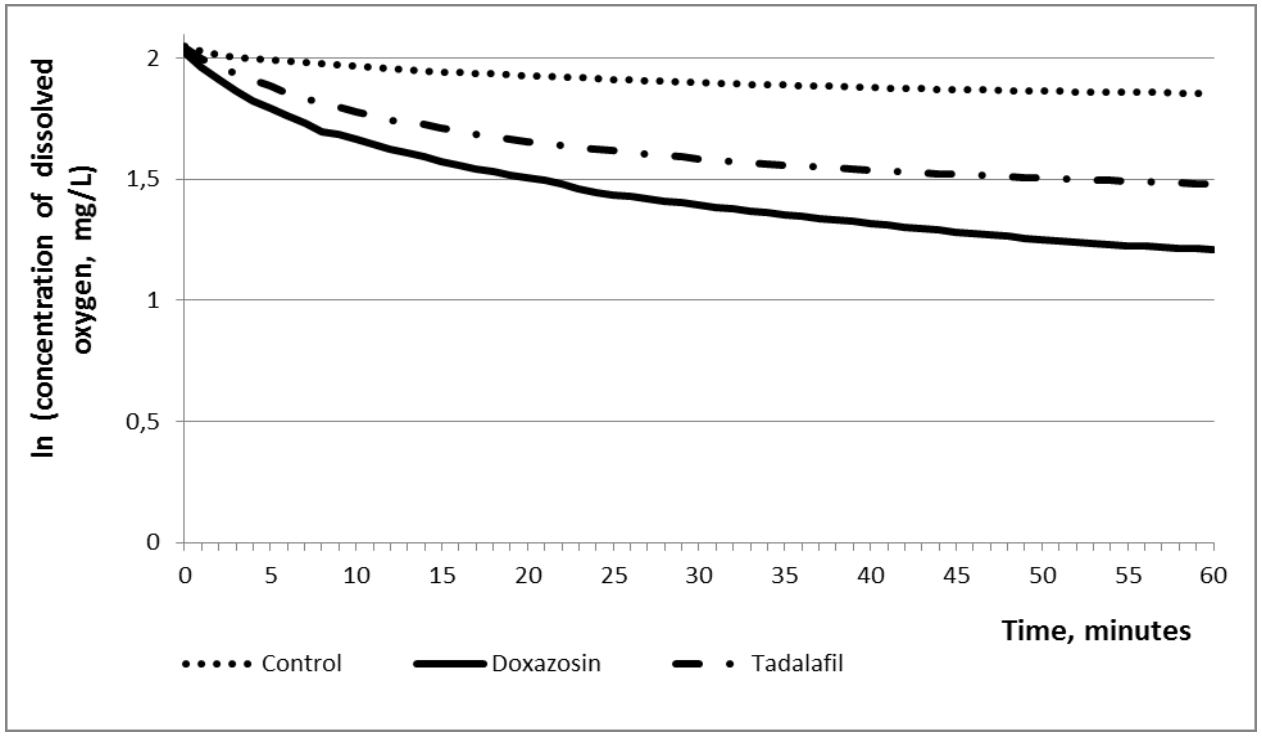

Figure 2. Doxazosin and tadalafil increase the rate of oxygen consumption by the tissue of prostate in white outbred rats.