Clinical and experimental rationale for antioxidant therapy of chronic bacterial prostatitis

Oleg I. Bratchikov¹, Igor A. Tyuzikov², Pavel A. Dubonos³

¹ Urology Department, Kursk State Medical University, 3 Karl Marx St., Kursk 305041, Russian Federation
² Tandem-Plus Medical Center, 3V Pervomayskiy Lane, Yaroslavl 150000, Russian Federation

Corresponding author: Oleg I. Bratchikov (bratov45@mail.ru)

Abstract

Introduction: Literature data prove the important role of oxidative stress in the pathogenesis of Chronic Bacterial Prostatitis (CBP) and its recurrence, which reduces the effectiveness of standard etiotropic therapy of the disease.

Aim of study: To improve the results of the pharmacotherapy of CBP by a comprehensive assessment of oxidative disorders in the prostate gland in a clinical and experimental study to provide evidence for antioxidant support.

Material and methods: The results of experimental simulation of CBP in 60 male rats and examination of 90 patients with CBP (average age 38.2 ± 1.4; main group) and 30 clinically healthy men (average age 35.5±1.5; control group), which included history-taking, collecting complaints, questioning, general and special examinations, biochemical, cytological, microbiological, sonographic studies. In some experimental animals and patients with CBP, different modes of pharmacotherapy were tested (antimicrobial monochemotherapy; antimicrobial chemotherapy+zinc picolinate; antimicrobial chemotherapy+L–carnitine tartrate in standard doses). The data were processed using descriptive and comparative statistics.

Results and discussion: Clinical and experimental findings showed the compensatory nature of the prostatic oxidative disorders after a standard antimicrobial monochemotherapy of the first episode of CBP and their continued persistence with a high risk of decompensation and development of mitochondrial dysfunction after a course of standard antimicrobial monochemotherapy in CBP recurrence. Zinc deficiency in the patients with CBP was detected on average 2.7 times more often than in the healthy men, so zinc determination in the prostatic fluid and subsequent drug compensation should be considered as first–line diagnostic and treatment measures. In the patients with CBP without zinc deficiency, L-carnitine may be an effective alternative to pharmacological correction of the prostatic oxidative disorders.

Conclusion: To increase the effectiveness of standard etiotropic therapy of CBP, simultaneous antioxidant support is necessary, using differentiated administration of antioxidants/antihypoxants (zinc or L-carnitine).

Keywords

Chronic bacterial prostatitis, experimental simulation, antimicrobial chemotherapy, free radical oxidation, lipid peroxidation, superoxide dismutase, succinate dehydrogenase, antioxidant, antihypoxant, zinc, L-carnitine.
**Introduction**

Chronic prostatitis (CP) is one of the most common urological diseases in men of different ages, but the results of its complex pharmacotherapy, even applying modern pharmacological achievements, in many cases can not be considered satisfactory, which results, first of all, in a low quality of life of this category of patients (Nickel and Weidner 2000; Loran et al. 2002; Kogan et al. 2009; Belousov et al. 2013; Dosta and Sevostianov 2013).

One of the key reasons for this situation is the complex etiology and multi-factor nature of the CP pathogenesis, including its infectious (bacterial) type – chronic bacterial prostatitis (CBP), which makes up 5–7% of the total structure of the disease types (Litwin et al. 1999; Engel et al. 2018; Grabe et al. 2018).

Currently, it is understood that, in the etiopathogenesis and outcomes of CBP, an important role is played not only by direct negative effects of pathogenic microorganisms that cause direct reactions of alterations and inflammatory infection in the prostate tissue (inflammatory infection mechanisms), but also by a cascade of secondary non-infectious inflammatory biochemical reactions, which are induced by the infectious matter and inevitably involved in pathogenetic interaction with it and have adaptive and reparative nature (Kullisaar et al. 2012; Tyuzikov et al. 2013; Aoun et al. 2015; Molochkov et al. 2015). The level of prostate cells protection from the free radical oxidation arising as a result of inflammatory infection, as well as its further development and especially the outcomes determined by a degree of severity of structural damage and functional deficiencies in the prostate tissue after eradication of the pathogen from it and clinical and laboratory relief of CBP exacerbation depends on the safety, adequacy and direction of these secondary adaptive reactions (Tyuzikov et al. 2013; Grabe et al. 2018).

One of the key natural mechanisms of cellular adaptive reactivity is the antioxidant support network (ASN), consisting of various enzymatic and non-enzymatic natural antioxidants and present in all cells of the human body without exception (Kostyk and Potapovich 2004; Mentschikova et al. 2006). An infectious agent in the prostate gland in CBP naturally leads to the primary activation of ASN in protective mechanisms, which are the main cellular organelles that resist oxidative stress, so a “biochemical scenario” of their function and direction of these secondary adaptive reactions (Tyuzikov et al. 2013; Grabe et al. 2018).

Currently, an active search for effective antioxidant drugs and rational modes of their administration in CBP is continuing (Balercia et al. 2017). However, according to the available Russian literature, a number of important practical issues of complex pharmacotherapy of CBP remain open, in particular, the sequence of applying etiotropic antibacterial and pathogenetic antioxidant therapies, the possibility of accurate laboratory diagnosis of deficiency/insufficiency of the most important natural antioxidants (for example, zinc and selenium) and the correlation of their plasma and prostatic concentrations, the definition of clear indications for the administration of the antioxidant depending on the clinical and laboratory features of CBP and a number of other treatment and diagnostic issues important for clinical practice.

**Aim of study**

Improving the results of the pharmacotherapy of Chronic Bacterial Prostatitis by a comprehensive assessment of the local oxidative disorders in the prostate gland in a

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**References**

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clinical and experimental study and to provide rationale for a differentiated combined etiopathogenetic pharmacotherapy with antioxidant support in this disease.

**Material and methods**

The study, which consisted of two parts – experimental and clinical, was organized and performed in accordance with the regulatory acts and guidelines governing the conduct of experimental and clinical research in the Russian Federation. Laboratory animals were treated in accordance with the current Rules for the Use of Experimental Animals, International Guiding Principles for Biomedical Research Involving Animals (1985) and Russian Guidelines for Experimental (Pre-clinical) Studies of New Pharmacological Agents (2000). All the patients who entered the main group of the clinical part of the study and completed it, as well as the men of the control group, had been previously informed of the aims and objectives of the study, and each of them filled in an informed consent for participation in the study and for using his personal results of the study for further statistical analysis.

The experimental part of study was performed in 60 outbred mature healthy male rats weighing 180–200 g, after a 14-day quarantine regime, without any signs of acute and chronic diseases. To simulate a laboratory model (CBP), a modified method by Nickel J. C.-Goto T. (1991) was used. The prostate gland and posterior urethra of male rats were infected by introducing a 0.05 ml and 0.1 ml of suspension of *E. coli NIHJ JC-2* culture at the concentration of 108 CFU/ml into the prostatic part of the urethra through a catheter. At the same time, 20 animals were infected once (experimental model of “CBP episode”), 20 animals were re-infected 30 days later (experimental model of “CBP recurrence”), and the remaining 20 intact animals made up the control group. After euthanasia, preparation and necessary studies of prostate homogenates of the laboratory animals were performed.

The clinical part was based on clinical observations, and pharmacotherapy of 90 men (average age 38.2±1.4) without prostate pathology. The total control group consisted of 30 clinically healthy men (average age 35.5±1.5) years; confidence interval 0.95|1–13). The frequency and main symptoms of CBP in the main group were taken as normal reference values of the studied indicators.

The main group of the study was ongoing, prospective and full-design. The results of the complex examination of men in the control group were taken as normal reference values of the studied indicators.

### Entry criteria:

- Presence of clinical CBP symptoms (the main clinical sign is chronic pelvic/prostatic pain with typical irradiation) in combination with appropriate laboratory validation (identification of significant pathogens in the prostatic fluid in a diagnostically significant titer >10^3 CFU/ml)
- Absence of a history of surgery or trauma of the pelvis and perineum
- No symptoms of any neurological disease
- No diabetes mellitus type 1 or type 2
- Absence of anamnesis and clinical and laboratory signs of infections (STIs) at the time of the study
- Age of men up to 50

### Exclusion criteria:

- Presence of any clinical and sonographic signs of infravesical obstruction of any genesis
- Presence of lower urinary tract symptoms (LUTS) typical for overactive bladder
- Therapy of any LUTS or chronic pain earlier than 3 months ago, without any positive results
- Taking medications that can affect the bladder and/or prostate less than 6 months before the start of the study
- Known or suspected prostate cancer (total blood PSA > 4 ng/ml)
- Individual drug intolerance or contraindications to the medications used in this study.

Statistical processing of the age index of the control group (n = 30) showed an average age of 35.5 ± 1.5 years (confidence interval 0.95|20–45), and for the patients of the main group (n = 90) the average age was 38.2 ± 1.4 years (confidence interval 0.95|24–46). Thus, the control and main groups were homogeneous in age, since the confidence intervals overlapped in each group. The minimum duration of CBP was 1 year, the maximum duration was 13 years (the average duration of CBP was 7.5 ± 1.4 years; confidence interval 0.95|1–13). The frequency and structure of the clinical symptoms of CBP in the main group are presented in Table 1.

| Clinical symptoms                                      | Absolute number (people) | Percentage (%) |
|--------------------------------------------------------|--------------------------|----------------|
| Pelvic/prostate pain syndrome with or without pain irradiation to adjacent anatomical regions | 90                       | 100.0          |
| Increased anxiety and depression levels                 | 38                       | 42.2           |
| Impaired productivity due to the chronic pain           | 36                       | 40.0           |
| Reduced sex drive                                       | 32                       | 35.5           |
| Reduced frequency and degree of morning and adequate erections | 31                       | 34.4           |
| Orgasmic disorders                                      | 21                       | 23.3           |
| Inappropriate urination                                 | 15                       | 16.7           |
Complaints and anamnestic data had been collected from all the men before the start of examination and treatment, in accordance with the generally accepted medical methods. To objectify and evaluate the severity of CBP and the quality of life of the patients after collecting anamnesis and complaints, a questionnaire was conducted using a valid questionnaire – NIH–CPSI–QL (National Institute of Health Chronic Prostatitis Symptom Index – Quality of Life). All men underwent general physical and special urological examination using standard methods. The laboratory examination of the prostatic fluid was performed using standard cytological and microbiological studies. To exclude concomitant sexually transmitted infections (STIs), urethral smears of all men in the main and control groups were examined using two methods: enzyme immunoassay (ELISA) and polymerase chain reaction (PCR). To determine the level of reactive oxygen species (ROS) in biological substances, the method of luminal-dependent chemiluminescence (LDCL) was used, based on the use of a mixture of a chemiluminescent probe – luminol (3-aminophthalhydrazide) – and horse-radish peroxidase for accurate measurements of hydrogen peroxide formation. Determination of lipid peroxidation products (LPP) in biological substrates was performed using the following methods: diene conjugates were determined by the method of Stalnaya I.D. (1977); malondialdehyde was determined using spectrofluorometry after its reacting with thiobarbituric acid by the method of Stalnaya I.D. and Garishvili T.G. (1977). Superoxide dismutase (SOD) activity was determined by a spectrophotometric method, using the method of Mistra H.P., Fridovich I. (1972) modified by Kostyuk V.A. et al. (1990). Succinate dehydrogenase activity was determined by the method of Storozhuk P.G. and Storozhuk A.P. (2004), based on the ability of this enzyme to restore nitroblue tetrazolium (NBT) to formazan. In order to exclude prostate cancer, all the men of both groups at the initial stage of the study were determined the blood level of total PSA by a heterogeneous two-stage enzyme immunoassay, using standard Enzymun-Test PSA assays by BoehringerMannheim Corp. (Germany). The zinc content in the blood serum was determined by colorimetric method (IFCC), based on the formation of a colored complex compound of zinc with dithizone. The content of zinc in the prostatic fluid was determined by X-ray fluorescence analysis, based on the spectrum features of secondary fluorescence radiation of the sample, which occurs under the influence of hard-er X-rays. To test the reference parameters of the healthy men (prostate volume and its structure), as well as to assess the initial morphometric parameters of the prostate and their dynamics during observation and pharmacotherapy, all men of the main and control groups included in the study were subjected to transrectal US by a 5.5–7-MHz rectal biplane sensor (Ultramark-9, USA) and an ultrasound complex Logiq 500 Pro Series (USA). To assess the effectiveness of CBP treatment in the experimental and clinical parts of the study, several pharmacotherapy modes were tested (antimicrobial monotherapies (levofloxacin); antimicrobial chemotherapy + zinc pico-linate; antimicrobial chemotherapy + L–carnitine tartrate in standard doses for 28 days). Statistical processing was performed in Microsoft Excel-2007 and Statistica 6.0. (StatSoft, USA). Data processing was performed using descriptive and comparative statistics. The results of the study were entered into a personal computer based on Microsoft Excel–2007 and Statistica 6.0. Spearman correlation coefficient (r) was determined to study the interaction among the quantitative features. The Student’s t-test was used to evaluate the intergroup differences in the values of indicators that have a continuous distribution. To solve the problems of studying the influence of two or more conditions on a certain random variable, various statistical methods of multivariate analysis were used. The critical confidence level of the null statistical hypothesis (about the absence of significant intergroup differences or factor influences) was assumed to be 0.05. The value of p<0.05 generally accepted in biomedical research was considered as statistically significant for all indicators.

Results and discussion

In the experiment with simulated CBP episode, in the prostrate tissue of the laboratory animals a significant increase in the processes of free radical oxidation was revealed (a significant 4.6-time increase in the number of ROSs, a 2-time increase in their activity, an increase in the level of intermediate lipid peroxidation products (diene conjugates and malondialdehyde) by 34.6% and 42.0%, respectively, a 1.5-time increase in the activity of superoxide dismutase (SOD) compared with those in the intact animals of the control group (p < 0.05), without significant dynamics of mitochondrial succinate dehydrogenase (SDH)). After the course of antimicrobial monotherapy, the number of ROSs in the prostate tissue was normalized, but their activity remained 1.6 time higher, the level of malondialdehyde was 20.1% higher and the activity of SOD – 19.7% higher, compared those of the control group (p < 0.05).

In the prostate gland of the laboratory animals with simulated CBP recurrence, significantly more pronounced cellular oxidative disorders were detected compared to the CBP episode model (a 7.6-time increase in the number of ROS, a 3.5-time increase in their activity, a 33.3% increase in SDH activity (p < 0.05) against the background of multidirectional changes in the level of lipid peroxidation products and a 32.0% lower SOD activity than in the control group (p < 0.05)). After a course of antimicrobial monotherapy, in the prostate tissue there remained significantly higher levels of ROS (3.45 times) and their activity (2.1 times) against a significantly lower (32.0%) level of SOD activity (p < 0.05) and unreliable lower (30.1%) level of mitochondrial SDH compared with those of the control group (p < 0.1).

The comparative clinical and laboratory characteristics of the control and main groups of the clinical part of the study are presented in Table 2.
As follows from Table 2, the differences in the clinical and laboratory indicators between the control and main groups were statistically significant (p < 0.05). In contrast to the healthy men of the control group, the patients with CBP had oxidative imbalance in the prostate gland (increased number and activity of ROS, increased leukocytosis of the prostatic fluid, and more lipid peroxidation and SOD activity in the prostatic fluid) and its secretory disorders (reduced number of lecithin granules in the prostatic fluid, the high frequency of the disorders of crystallization of the prostatic fluid), which, from the clinical point of view, corresponded to more pronounced symptoms of pain syndrome and more inferior quality of life in the patients with CBP compared with the healthy men in the control group (p < 0.05). In the patients with CBP, significant positive correlations were found between the amount of ROS and SOD activity in the prostatic fluid (n = 90; r = 0.413; p = 0.001) and between the amount of ROS in the prostatic fluid and the clinical pain index (n = 90; r = 0.304; p = 0.001).

The frequency of the absolute serum zinc deficiency in the patients with CBP was 28.9%, which is 2.89 times significantly higher than in the control group of healthy men (10.0%, respectively; p < 0.05). The frequency of the absolute zinc deficiency in the prostatic fluid in the patients with CBP was 41.1%, which is 2.5 times significantly higher than in healthy men of the control group (16.7%, respectively; p < 0.05). In general, the healthy men of the control group had a statistically significant weak positive correlation between the zinc levels in the serum and the prostatic fluid (r = 0.156; n = 30; p = 0.001), which turned out to be more statistically strong (r = 0.204; n = 7; p = 0.001) in the range of subnormal values (< 543 mcg/L) and the lower tercile of the reference normal values of serum zinc level (543–738 mcg/L). A statistically significant moderate positive correlation (r = 0.345; n = 37; p = 0.001) was also found in the patients with CBP with absolute zinc deficiency and lower limit of normal serum levels. The patients with CBP with zinc deficiency compared to the patients with CBP without it showed non-significantly worse clinical characteristics of pain and quality of life, non-significantly higher levels of leukocytosis, malondialdehyde and a lower content of lecithin granules in the prostatic fluid (p < 0.1), but significantly lower (by 20.2%) activity of SOD in the prostatic fluid (p < 0.05), between the activity of which and the concentration of zinc in the prostatic fluid a significant moderate positive connection was revealed (r = 0.389; n = 90; p = 0.001).

The integrative results of comparative evaluation of the effectiveness and tolerability of the CBP pharmacotherapy modes tested in this study are presented in Table 3. As follows from Table 3, the standard antimicrobial monochemotherapy mode in contrast to the modes of combined pharmacotherapy with additional prescription of zinc and L-carnitine showed significantly worse results of microbiological eradication of pathogens and treatment in relation to the clinical characteristics of the disease, the quality of patients’ life, secretory function of the prostate gland and especially its oxidative status (p < 0.05), which violations in the form of a hyperproduction of ROS and accumulation of the lipid peroxidation products (malondialdehyde) – along with a decreased SOD activity in the prostatic fluid continued to persist after a course of antimicrobial monochemotherapy.

Thus, the experimental findings showed the compensatory nature of oxidative changes in the prostate cells after standard antimicrobial monochemotherapy of the first episode of CBP (ASN compensation phase) and the continuing persistence of the oxidative disorders in the prostate cells after a course of standard antimicrobial monochemo.

therapy with a high risk of mitochondrial dysfunction in CBP recurrence (ASN decompensation phase).

The obtained results of the clinical part of the study also confirmed the negative impact of the infectious agent on the oxidative status of the prostate gland and reflected a significant role of free-radical prostatic aggression as an additional non-infectious component in the pathogenesis of pain syndrome in CBP.
Compared with the healthy men, the patients with CBP were on average 2.7 times more likely to have a deficiency of serum and/or prostatic zinc. Against the background of this, they had worse clinical and laboratory parameters of the disease and persisting oxidative disorders in the prostatic fluid in contrast to the patients with CBP without zinc deficiency. In routine clinical practice, direct zinc determination in the prostatic fluid should be considered as the most objective and informative method of zinc deficiency diagnosing in patients with CBP. Standard etiotropic antimicrobial chemotherapy of CBP does not guarantee a complete pharmacological cure of the prostate and has no affect on the course and outcomes of free-radical oxidation, which naturally develops against the background of the entry of an infectious agent into the prostate tissue. In this regard, additional prescription of medicinal agents that can safely and effectively neutralize the negative impact of oxidative stress on the prostate gland (antioxidants and/or antihypoxants) is pathogenetically substantiated to improve the results of modern CBP pharmacotherapy.

Taking into account the crucial physiological role of zinc in prostate metabolism and the high frequency of zinc deficiency in patients with CBP, the first-line therapy in zinc-deficient patients with CBP is a medical correction of the deficiency of this essential micro-element. For patients with CBP without zinc deficiency, the administration of L-carnitine tartrate may be an effective and safe front-line therapy to correct the existing oxidative disorders in the prostate gland. The study showed a significant negative role of free-radical reactions in the formation of anatomical and functional disorders in the prostate gland in CBP, which are able to complete the "vicious loop" of its pathogenesis and maintain the organ alterations after a course of standard antimicrobial monochemotherapy (Fig. 1).

Based on the results of the study, the following practical algorithm for zinc deficiency diagnosing in patients with CBP is proposed (Fig. 2.).

An optimized algorithm for the sequence and differentiated administration of combined etiopathogenetic pharmacotherapy in CBP, based on the results of the study, can be presented as follows (Fig. 3).

The proposed practical algorithms are designed to improve the traditional diagnostic and pharmacotherapy of CBP carried out in a routine urological use and to implement an individual approach to the management of patients with this disease.

**Conclusion**

The obtained results of the clinical and experimental study convincingly confirmed the significant negative role of free radical oxidation (oxidative stress) induced by an infectious agent in the multifactorial pathogenesis of CBP, which has been reflected in the scientific literature of recent years. In this connection, it is possible to substantia-
Figure 1. The role of oxidative local disorders in the prostate gland in the formation of the “vicious loop of pathogenesis” of CBP. Abbreviations: LP – lipid peroxidation; SOD – superoxide dismutase; SDG – succinate dehydrogenase; ASN – antioxidant support network.

Figure 2. Diagnostic algorithm for zinc deficiency detecting in CBP patients in routine urological use.
Figure 3. Algorithm of the sequence and differentiated choice of personalized combined etiopathogenetic pharmacotherapy in patients with CBP.

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Author contributions

Oleg I. Bratchikov, Doctor of Medical Sciences, Professor, Head of the Department of Urology, e-mail: bratchikov45@mail.ru The author was engaged in research design, analysis of the obtained data, and the article-writing

Igor A. Tyuzikov, Doctor of Medical Sciences, Professor, urologist, e-mail: phoenix-67@list.ru The author obtained the data for the analysis, analyzed the obtained data, and was engaged in the article writing.

Pavel A. Dubonos, Postgraduate Student, Department of Urology, e-mail: v-utkin@rambler.ru The author reviewed the relevant literature and obtained the data for analysis.