EXPERIMENTAL STUDY OF THE NONSTERoidal ANTI-INFLAMMATORY DRUGS APPLICATION UNDER USING LOW-INTENSITY INFRARED LASER RADIATION

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Topicality. The combined application of nonsteroidal anti-inflammatory drugs (NSAIDs) and low-intensity infrared laser radiation (LIIRLR) for treatment of osteoarthrosis has remained an actual task.

Aim. To study the NSAIDs usage under LIIRLR application in experiments in rats.

Materials and methods. The time for LIIRLR application (15 min after drug administration) was chosen according to our previous studies on diclofenac sodium (DNa) pharmacokinetics in rats blood plasma. To study the NSAIDs application at the influence of LIIRLR, white beardless male rats (n = 15) of 250-300 g were used in the experiments. The animals were divided into 3 groups. Rats of group 1 received purified water (per os) and LIIRLR. Rats of group 2 received DNa at a dose of ED50 (per os, 8 mg/kg). Rats of group 3 were influenced of LIIRLR and in 15 min received DNa (per os, 8 mg/kg). For DNa extraction from rat plasma samples, the method of solid-phase extraction was used. Concentration of DNa was determined by method of high-performance liquid chromatography (HPLC).

Results and discussion. It was established that at the combined use LIIRLR and DNa the drug concentration in the rats blood serum was 1.7 fold higher in comparison to DNa application alone.

Conclusions. It is proved, that the method of combine application of LIIRLR and NSAIDs (in 15 min) was more effective than the use of NSAIDs alone.

Key words: osteoarthrosis; nonsteroidal anti-inflammatory drugs; diclofenac sodium; glucosamine; low-intensity infrared laser radiation; lasertherapy; high-performance liquid chromatography
INTRODUCTION

Diseases of musculoskeletal system (MSS) and connective tissue are widespread in many countries of the world; they lead to decreased working capacity, worsening of the quality of life, social disadaptation, and, in the consequence, disability. Osteoarthritis (OA) is one of the central positions in therapy of these diseases and are most widely used in modern medicine, clinical practice, and pharmacy [2, 4].

Diclofenac sodium (DNa) remains the reference drug of NSAIDs group [1, 3, 4]. However, in spite of a great number of treatment schemes used, pharmacological correction of MSS and connective tissue diseases is an important and urgent task, which calls for new methods of treatment [1, 2].

There are various drugs and methods of treatment for MSS and connective tissue diseases. Nonsteroidal anti-inflammatory drugs (NSAIDs) constitute one of the central positions in therapy of these diseases and are used in medicine, clinical practice, and pharmacy [2, 4].

It has been found that physical factors can potentiate drugs' action. Due to this fact, use of low-intensity infrared laser radiation (LIIRLR) attracts the attention of scientists. This method of treatment has analgesic, anti-inflammatory, pain alleviating, regenerating, desensitization, bacteriostatic effects; it also improves local blood circulation [1, 4, 5].

Thus, the possibility of combined use of NSAIDs and LIIRLR for treatment of MSS and connective tissue diseases is an urgent and well-grounded task; it will allow to improve the results of treatment of OA patients.

The aim of this work is to study NSAIDs use along with LIIRLR in the experiment in rats.

MATERIALS AND METHODS

Diclofenac sodium (DNa), sodium 2-[(2,6 – dichlorophenyl) amino] phenyl) acetate, has marked anti-inflammatory, analgesic, as well as moderate antipyretic activities [3]. Substance of DNa manufactured by Borshchakivskyi Chemical-Pharmaceutical Plant (BCPP), Ukraine (batch No.20040609) was used for the study. Substance of DNa manufactured by “Amoli Organics Ltd” (batch No. 20061013) was used as a working reference standard (WRS).

Identification and quantitative determination of DNA in the substance of DNa were carried out by most sensitive method of high-performance liquid chromatography (HPLC) [6, 7]. This method was used to determine concentrations of the test substance at all stages of the study.

Animals used in the experiment: white beardless male rats of the population of experimental biological clinics of the State Enterprise “Sytenko Institute of Spine and Joint Pathology of the National Academy of Medical Sciences of Ukraine”.

Conduct of experiments was approved by the local committee on bioethics of the State Enterprise “Sytenko Institute of Spine and Joint Pathology of the National Academy of Medical Sciences of Ukraine” (protocols No. 44 dated October 22, 2007; No. 81 dated December 20, 2010).

Work with animals was carried out according to Directive of the European Parliament and European Council 2010/63 EU.

The time of LIIRLR use and the time of animal sacrifice have been determined in our previous studies on pharmacokinetics of DNa in rat plasma by method of high-performance liquid chromatography (HPLC) [8].

LIIRLR was carried out in 15 min after drug administration with laser therapeutic unit “Mustang” set as follows: wavelength is 0.89 µm, pulse power is 7-8 W, pulse frequency is 3.000 Hz, duration of the session is 3 min 42 sec, radiation dose is 0.3 J (calculated by specialists of National Research Center “Metrology Institute”, Kharkiv, Ukraine). The apparatus was used by contact along the posterior surface of the rat knee joint with preliminary removed hair [9].

To study concomitant use of NSAIDs and LIIRLR, we selected 15 male rats with body weight of 250-300 g, which were kept according to according to sanitary norms on a standard diet [10]. Experimental animals were subdivided into 3 groups, 5 rats per group. DNa was diluted in purified water and administered in the same volume at a dose of ED_{50} (per os, 8 mg/kg of animal body weight). Animals of group 1 received purified water (per os, 0.5 mL per 100 g of animal body weight) and were exposed to LIIRLR. Animals of group 2 received per os DNa at a dose of ED_{50} [10]. Animals of group 3 were subjected to the influence of LIIRLR and in 15 min received DNa (per os, 8 mg/kg). Animals were sacrificed in 60 min after drug introduction [8].

Blood was sampled in the quantity of 7-10 ml into labeled test-tubes that were heparinized. Blood samples were centrifuged (3.000 rpm, 15 min), and plasma was obtained. The interval between blood sampling and its processing did not exceed 5 min. Before analysis, plasma samples were kept at – 80 ºC.
For extraction of DNA from rat plasma samples, a method of solid-phase extraction was used [7]. Cartridges Supel™-Select HLB SPE (Supelco) 30 mg/1 ml were used for work. The method had been modified due to micro-concentrations of the active substance during previous stages of the complex study [11].

Statistical processing of experimental data obtained was carried out with software STATISTICA (StatSoft Inc., the USA). Reliability of the results obtained was assessed at the level of significance not less than 95% (p ≤ 0.05) [12].

RESULTS AND DISCUSSION

Confirmation and determination of quantitative determination of DNA (manufactured by BCPP) in rat plasma were carried out in comparison to DNA WRS manufactured by “Amoli Organics Ltd”. Chromatograms of DNA substance samples obtained under the same conditions are presented in Fig. 1.

Retention time of DNA in the chromatogram of the test sample of the solution (5.673 min) corresponds to that of DNA in the chromatogram of DNA WRS (5.673 min), so the sample provided is DNA substance. In the result of the studies conducted, quantitative content of DNA and suitability of the dose studied. Actual dose of DNA during study conduct is 8 mg/kg.

Chromatogram of the control solution (rat’s blood plasma), which were exposed to LIILRLR is shown in Fig. 2.

When studying plasma concentration of DNA in animals of group 2, which received DNA per os (8 mg/kg), it was determined to be 2.36 ± 1.10 µg/mL. Plasma concentration of DNA in animals of group 3 subjected to the influence of LIILRLR and in 15 min received DNA per os (8 mg/kg) was 4.08 ± 1.96 µg/mL. Typical chromatograms of the test solution and reference solution are presented in Fig. 3, 4.

Thus, quantitative content of DNA in the substance of DNA and suitability of the dose studied (8 mg/kg) were confirmed. It was established that under the scheme of the combined use of LIILRLR and DNA (per os, in 15 min) in comparison to the group of animals with DNA (per os) administration, the concentration of drug in blood plasma of rats was increased in 1.7 times. This shows the effectiveness of the combined use of LIILRLR and NSAIDs.

The results obtained indicate to the fact that concomitant use of NSAIDs and LIILRLR potentiate the ac-

![Fig. 1. Chromatograms of diclofenac sodium substance samples. 1 – chromatogram of DNA manufactured by “Amoli Organics Ltd”; 2 – chromatogram of DNA used in the experiment as WRS manufactured by BCPP](image1)

![Fig. 2. Chromatogram of the control solution (blood plasma of rats, which were exposed to LIILRLR)](image2)
tion of drugs. This allows to decrease the dose, influence intake and duration of NSAIDs’ action in the organism, decrease the frequency of their administration, and is a subject for further study.

CONCLUSIONS

Use of NSAIDs along with LIIRLR has been studied in experiments on rats. It is proved, that the method of combined application of LIIRLR and NSAIDs (per os, in 15 min) was more effective than the use of NSAIDs alone. The scheme of concomitant indication of LIIRLR and NSAIDs studied will be used for further preclinical and clinical studies, as well as for improvement of the quality of treatment in osteoarthrosis patients.

Conflicts of Interest: authors have no conflict of interest to declare.

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