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Laser Doppler flowmetry for studying tissue blood flow in a model of carrageenan-induced edema in mice

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Abstract. ***Introduction.*** Carrageenan-induced edema is the most common *in vivo* model for evaluating the therapeutic efficacy of anti-inflammatory drugs. The pathogenesis of carrageenan-induced edema is based on acute exudative inflammation, in which microcirculation disorders play an important role. The study of the pathogenesis of microcirculation is of great interest, both diagnostic and prognostic, in assessing the functional status of the organism. One of the promising and successfully applied diagnostic methods for studying tissue blood flow is laser Doppler flowmetry, which makes it possible to assess changes in microcirculatory processes. ***The aim*** is to evaluate tissue blood flow in mice using the laser Doppler flowmetry method in comparison with plethysmometry parameters in the carrageenan-induced edema model to study the efficacy and pharmacodynamics of biomedical cellular products and pharmaceutical substances in preclinical practice. ***Materials and methods.*** 40 mice (20 males and 20 females) of the BALB/c line aged 8–10 weeks were used in the study. During the experiment, carrageenan-induced edema was modeled, laser Doppler flowmetry and plethysmometry were performed in the area of the left hind limb of the experimental animal. According to the results of the conducted research, the following tasks were performed: 1) assessment of tissue perfusion in mice in the area of plantar aponeurosis in dynamics; 2) plethysmometry in dynamics (assessment of the severity of edema in animals by changes in the volume of the animal's paw); 3) comparison of plethysmometry and laser Doppler flowmetry. It was found that the degree of blood flow restoration and the severity of edema correlate with each other (the correlation coefficient is 96%). ***Conclusions.*** The use of laser Doppler flowmetry in the carrageenan-induced edema model in mice makes it possible to assess the state of microcirculation *in vivo* and dynamically. The method is characterized by accuracy and high sensitivity, which makes it possible to determine changes in blood microcirculation in the early stages, including before the "visual" manifestation of edema. At the same time, there is a high correlation between the data obtained using laser Doppler flowmetry and plethysmometer. In the future, this method can be widely used in preclinical practice to evaluate the therapeutic efficacy and pharmacodynamics of drugs under development that affect regional blood circulation and microcirculation.

Keywords: carrageenan-induced edema, laser Doppler flowmetry, LDF, biological test system

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Применение лазерной допплеровской флоуметрии для исследования тканевого кровотока на модели каррагинан-индуцированного отека у мышей

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Резюме*. Введение.* Каррагинан-индуцированный отек — наиболее распространенная модель *in vivo* для оценки терапевтической эффективности противовоспалительных лекарственных препаратов. В основе патогенеза каррагинан-индуцированного отека лежит острое экссудативное воспаление, в котором важную роль играет нарушение микроциркуляции. Изучение патогенеза микроциркуляции представляет большой интерес, как диагностический, так и прогностический, при оценке функционального статуса организма. Одним из перспективных и успешно применяемых диагностических методов исследования тканевого кровотока является лазерная допплеровская флоуметрия, которая позволяет оценить изменения микроциркуляторных процессов. ***Цель*** — провести оценку тканевого кровотока в динамике у мышей методом лазерной допплеровской флоуметрии в сопоставлении с показателями плетизмометрии в модели каррагинан-индуцированного отека для изучения эффективности и фармакодинамики биомедицинских клеточных продуктов и фармацевтических субстанций в доклинической практике. ***Материалы и методы.*** В работе использовали 40 мышей (20 самцов и 20 самок) линии BALB/c в возрасте 8–10 недель. В процессе эксперимента моделировали каррагинан-индуцированный отек, проводили лазерную допплеровскую флоуметрию и плетизмометрию в области левой задней конечности экспериментального животного. По результатам исследования выполнены: 1) оценка тканевой перфузии у мышей в области подошвенного апоневроза в динамике; 2) плетизмометрия в динамике (оценка степени выраженности отека у животных по изменению объема лапы животного); 3) сопоставление показателей плетизмометрии и лазерной допплеровской флоуметрии. Установлено, что степень восстановления кровотока и выраженность отека коррелируют между собой (коэффициент корреляции составляет 96%). ***Выводы.*** Лазерная допплеровская флоуметрия в модели каррагинан-индуцированого отека у мышей позволяет прижизненно и в динамике оценить состояние микроциркуляции. Метод отличается точностью и высокой чувствительностью, с его помощью можно определить изменения микроциркуляции кровотока на ранних этапах, до «визуального» проявления отека. Наблюдается высокая корреляция между данными, полученными при использовании лазерной допплеровской флоуметрии и плетизмометра. В перспективе данный метод может быть широко применен в доклинической практике для оценки терапевтической эффективности и фармакодинамики разрабатываемых препаратов, влияющих на регионарное кровообращение и микроциркуляцию.

Ключевые слова: каррагинан-индуцированный отек, лазерная допплеровская флоуметрия, ЛДФ, биологическая тест-система

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

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INTRODUCTION

The microcirculatory bed is a trophic component of bloodstream circulation. It indirectly reflects tissue viability, as well as an ability to regenerate, perform immune functions, and carry out many other physiological functions that are essential for healthy tissues. Impaired tissue perfusion indicates more than just damage to a specific organ or tissue. It often results from systemic processes that lead to serious consequences. Understanding how microcirculation is affected by inflammation is important in preclinical research for evaluating the therapeutic efficacy of anti-inflammatory drugs [1–5].

One of the most common models of in vivo inflammation is carrageenan-induced edema. The pathogenesis of carrageenan-induced edema is characterized by acute exudative inflammation. Microcirculation disorders, accompanied by exudation and cell emigration, are considered one of its key components. Laser Doppler flowmetry (LDF) serves as a concise method for diagnosing microcirculatory disorders and has been attracting sustained interest from researchers worldwide for more than a decade [5, 6].

This method has been successfully used to study tissue microcirculation in humans; however, it is not currently widely applied in animals [2, 4, 7, 8].

Thus, LDF remains a promising area of research in preclinical practice.

AIM

The aim of the research was to evaluate dynamic tissue blood flow in mice using LDF in comparison with plethysmography parameters in a carrageenan-induced edema model. Thus, the study aimed to investigate the efficacy and pharmacodynamics of biomedical cell products and pharmaceutical substances in preclinical practice.

MATERIALS AND METHODS

Animals. The experiment involved 40 BALB/c mice (20 males and 20 females) aged 8–10 weeks, SPF-grade, obtained from the “Laboratory Animal Breeding Facility” of the Branch of the M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Pushchino town. Animal housing, care, and experimental procedures were conducted in accordance with international European bioethical standards, Russian ethical standards for the housing and handling of laboratory animals, and the norms and rules specified in the “Animal Use Policy of JSC ‘GENERIUM’.”

Study design. Two groups of animals were established: Group 1 (n=20: 10 females and 10 males) was used to model carrageenan-induced edema; Group 2 (n=20: 10 females and 10 males) was used to model carrageenan-induced edema + intramuscular administration of diclofenac, 25 mg/mL (Hemofarm, Serbia), administered as a single dose one minute after the induction of carrageenan-induced edema, at a dose of 3 mg/kg.

The experiment consisted of three stages: *Stage I* — induction of carrageenan-induced edema; *Stage II* — LDF; *Stage III* — assessment of the severity of edema in animals by plethysmography based on changes in paw volume.

Stage I. Modelling carrageenan-induced edema

When modeling carrageenan-induced edema, a 1% carrageenan solution (λ Carrageenan plant mycopolysaccharide (SIGMA-ALORICH/LOT:1408463V)) was administered subplantarly once into the animal’s left hind limb beneath the plantar aponeurosis in a volume of 25 μL (Fig. 1, a). The 1% carrageenan solution was prepared according to the method described by E. Phyllis et al. [9].



а/a *б/b*

Fig. 1. **Modeling of carrageenan-induced edema and assessment of microcirculation by laser Doppler flowmetry method: *a* — administration of 1% carrageenan solution (3 and 4 — plantar tubercules); *b* — laser Doppler flowmetry (photo by the authors)**

Рис. 1. Моделирование каррагинан-индуцированного отека и оценка микроциркуляции методом лазерной допплеровской флоуметрии: *а* — введение 1% раствора каррагинана (1 и 4 — подошвенные бугорки); *б* — лазерная допплеровская флоуметрия (фото авторов)

Stage II. Laser Doppler Flowmetry

Tissue perfusion was measured on the animal's left hind limb in the region of the plantar aponeurosis [10].

LDF was performed using a TSD145 laser Doppler sensor (BIOPAC Systems Inc., USA) and AcqKnowledge® 4 software.

For each time point, measurements were taken three times within one minute for each animal, and the average value of three measurements was recorded (Fig. 1, b).

Layout of the laser Doppler flowmetry recording setup

1. "0" time point — before administration of the 1% carrageenan solution.

2. "5 minutes," "10 minutes," "15 minutes," "30 minutes," "1 h," and "3 h" time points - 5, 10, 15, and 30 minutes, 1 hour, and 3 hours after administration of the 1% carrageenan solution.

The "0" measurement was performed on the hind limbs of animals in all experimental groups. No statistically significant differences were found between the left and right hind limbs. Subsequent LDF recordings were performed on the left hind limb. When analyzing the LDF trace, capillary blood flow was calculated to characterize the integrity of microcirculation at all time points of tissue perfusion recording relative to the baseline ("0" point).

Stage III. Assessment of edema severity in animals using a plethysmograph based on changes in paw volume

The severity of edema in animals was assessed using a plethysmograph (Ugo Basile Biological Research Apparatus, Italy) based on changes in the volume of the animal's left hind limb, according to the method described by C.J. Marris [11].

The paw volume was measured three times at each time point for each animal, and the average of the three measurements was calculated.

Scheme for assessing the severity of edema

1. "0" time point — before administration of the 1% carrageenan solution.

2. "1 h" and "3 h" time points — 1 and 3 hours after administration of the 1% carrageenan solution.

Limb volume at the "0" time point was assessed in the hind limbs of animals in all experimental groups. No statistically significant differences were found between the left and right hind limbs. Subsequent volume assessments were performed on the left hind limb.

After measuring limb volume, the edema index (EI) was calculated using the formula:

$$EI = 100 \cdot (V_t - V_0) / V_0,$$

where V_0, mL — paw volume before administration of *the* 1% carrageenan solution; V_T, mL — the volume of the paw 1 hour and 3 hours after the administration of a 1% carrageenan solution.

Statistical analysis of the results. The data obtained were tested for normality of distribution. Statistical analysis included calculation of the mean and standard deviation. To compare the values, the parametric test (Student's t-test) was used, since the sample corresponded to a normal distribution. Differences were considered statistically significant at $p < 0.05$. Statistical analysis of the data was performed using the IBM SPSS Statistics software package (version 23, IBM Corp., USA).

RESULTS AND DISCUSSION

The results of tissue perfusion in the plantar aponeurosis region of mice in a carrageenan-induced edema model, showed a statistically significant decrease in microcirculation at all measurement time points after the administration of a 1% carrageenan solution relative to the "0" time point in all animal groups. After carrageenan injection, all animal groups showed a 10–12% preservation of microcirculation by the 5th minute, which is typical for the stage of transient vasospasm. By the 10th minute, a marked increase in capillary blood flow (arterial hyperemia) was recorded in mice, with microcirculation preserved at 56% relative to the "0" point. At the 15th minute, a state of venous hyperemia was observed, with microcirculation preserved at 30% of baseline. When modeling carrageenan-induced edema based on LDF parameters at the "5 minutes," "10 minutes," and "15 minutes" time points, no statistically significant differences were detected within the experimental groups. At the "30 minutes," "1 h," and "3 h," when microcirculation parameters were recorded, a statistically significant improvement in blood flow was observed in animals in the second experimental group compared to the first group. At the "30-minute" time point, blood flow was preserved at 25% in the first group and at 36% in the second group. At the "1 h" and "3 h" time points, the level of microcirculation in the first group was 20% and 15%, respectively, and in the second group, 45% and 55%, respectively (Fig. 2).

According to plethysmography data, 1st group demonstarted increased edema in the experimental limb at 1–3 h time point which ammounted to 46–51% compared to "0" baseline. The peak swelling was observed at 3 h (51%). The degree of edema in Group 2 at 1–3 hours was 36–25% of the normal value (the peak degree of edema was recorded at 1 hour — 36%) (Fig. 3).

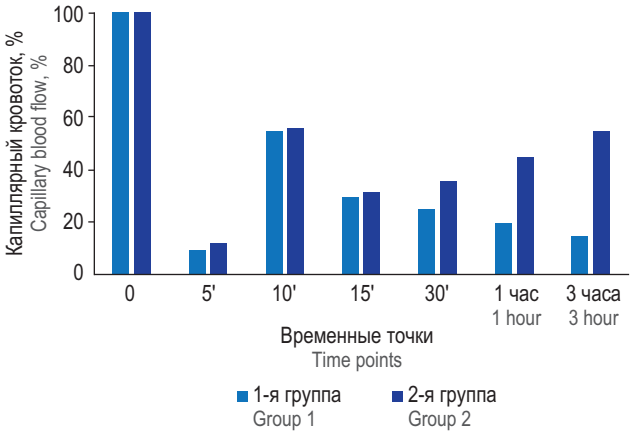


Fig. 2. **Dynamics of changes in capillary blood flow (according to laser Doppler flowmetry data), %. Group 1 — 1% carrageenan solution; Group 2 — 1% solution of carrageenan + Diclofenac, 3 mg/kg (figure by the authors of this article)**

Рис. 2. Динамика изменения капиллярного кровотока (по данным лазерной допплеровской флоуметрии), %. 1-я группа — 1% раствор каррагинана; 2-я группа — 1% раствор каррагинана + диклофенак в дозе 3 мг/кг (рисунок авторов данной статьи)

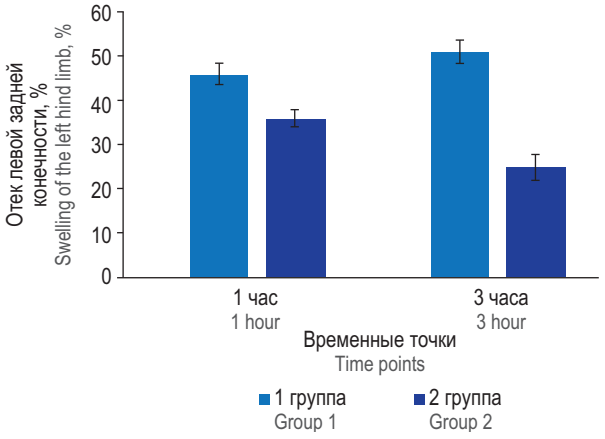


Fig. 3. **The severity of edema of the left hind limb (by volume; according to plethysmography), %. Group 1 — 1% carrageenan solution; Group 2 — 1% solution of carrageenan + Diclofenac, 3 mg/kg (figure by the authors of this article)**

Рис. 3. Степень выраженности отека левой задней конечности (по объему; по данным плетизмографии), %. 1-я группа — 1% раствор каррагинана; 2-я группа — 1% раствор каррагинана + диклофенак в дозе 3 мг/кг (рисунок авторов данной статьи)

Correlation analysis revealed that the degree of blood flow recovery and the severity of edema correlate with each other (correlation coefficient — 96%).

According to published research on carrageenan-induced edema, maximum paw edema in mice following carrageenan induction was observed after 3–5 hours and amounted to 41% of the initial paw volume, as measured by the plethysmographic method [9]. The plethysmography method is widely used and is highly accurate. However, it does not allow to assess microcirculation in this model, although this is the primary criterion for evaluating the anti-inflammatory properties of drugs in a number of studies. In addition to plethysmography, the severity of edema is assessed using the paw amputation method. However, this method yields data from a single animal at only one point, and it is technically difficult to apply to mice [12]. Currently, according to the literature, the most promising diagnostic approach for determining tissue inflammation in experimental models such as cutaneous neurogenic inflammation [6, 7, 13] and psoriatic arthritis [12] is the assessment of microcirculation. This method is based on measuring microcirculation in rats in the plantar aponeurosis region using LDF. This method allows not only for identifying individual variability in microcirculation but also for investigating the mechanisms of its regulation [4, 13].

Thus, assessing microcirculation using LDF in a carrageenan-induced edema model in mice to determine the degree of inflammation over time is a highly promising method. When compared with the plethysmography, LDF allows for in vivo and dynamic assessment of microcirculation status. It also provides a preliminary indication of the effectiveness of initiating therapy with anti-inflammatory drugs under development. At the same time, a high level of correlation is observed between the data obtained using LDF and a plethysmograph (the primary method for assessing the severity of edema in biological test systems).

CONCLUSION

Thus, based on results obtained in a mice model of carrageenan-induced edema, dynamic assessments of tissue blood flow were performed using the LDF method, and the resulting data were compared with plethysmography measurements.

It was established that LDF:

1) enables the assessment of microcirculatory status at different stages of carrageenan-induced edema with a high correlation between data sets;
2) is characterized by high accuracy and sensitivity, which allows to determine changes in microcirculatory blood flow at early stages, before the “visual” manifestation of edema.

Based on the above, this method can be used in preclinical practice to evaluate the therapeutic effects of drugs under development that affect regional blood

circulation and microcirculation, as well as to facilitate early diagnosis.

ADDITIONAL INFORMATION

Author contribution. E.V. Belyanina — development of the research concept, conducting the experimental study, analysis of the obtained results, writing the original text of the article; A.A. Ovsepyan — consultation on the research concept, scientific editing of the article; M.S. Aksenova — statistical processing of the results; Yu.V. Ishkova, Yu.S. Arilina, V.N. Garanova — assistance in conducting the experimental study; N.V. Belskaya — scientific and technical editing of the article; M.V. Lykov — consultation on the research concept, scientific and technical editing of the article.

All authors read and approved the final version before publication.

Competing interests. The authors declare that they have no competing interests.

Experiments with animals were carried out in accordance with international rules (Directive 2010/63/EU of the European Parliament and of the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes).

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