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DOSE-DEPENDENT FEATURES OF KINETIC CURVES OF CAFFEINE IN URINE OF LABORATORY ANIMALS

Research article

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Abstract

Currently, clearance tests are an important and integral component of assessing the liver functional state. They are a set of methods that allow you to determine the rate at which a certain substance is excreted from the body through the liver.

As a result of the experiment, the base and maximum levels of caffeine in laboratory animals were determined in order to further study its changes during the artificial induction of various types of hepatopathy in a particular experimental group of laboratory animals. These data provide an opportunity to study changes in caffeine levels in experimental hepatotoxicity states in the same experimental animals. The non-linearity of the caffeine biotransformation process at doses of 100 mg/kg and 150 mg/kg indicates the need to use the 3 dosage steps described in this article in subsequent experiments, and the dynamics of caffeine biotransformation will serve as a diagnostic criterion.

Keywords: clearance test, caffeine, liver pathology, laboratory animals.

ДОЗОЗАВИСИМЫЕ ОСОБЕННОСТИ КИНЕТИЧЕСКИХ КРИВЫХ КОФЕИНА В МОЧЕ ЛАБОРАТОРНЫХ ЖИВОТНЫХ

Научная статья

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Аннотация

В настоящее время клиренс-тесты являются важным и неотъемлемым компонентом оценки функционального состояния печени. Они представляют собой комплекс методов, позволяющих определить скорость выведения того или иного вещества из организма через печень.

В результате эксперимента были определены базовый и максимальный уровни кофеина в организме лабораторных животных с целью дальнейшего изучения его изменений при искусственной индукции различных видов гепатопатии у конкретной экспериментальной группы лабораторных животных. Эти данные дают возможность изучить изменения уровня кофеина при экспериментальных состояниях гепатотоксичности у тех же экспериментальных животных. Нелинейность процесса биотрансформации кофеина в дозах 100 мг/кг и 150 мг/кг указывает на необходимость использования в последующих экспериментах трёх ступеней дозирования, описанных в данной статье, а динамика биотрансформации кофеина будет служить диагностическим критерием.

Ключевые слова: клиренс-тест, кофеин, патологии печени, лабораторные животные.

Introduction

One of the most promising methods for diagnosing and differential diagnosing pathologies of the hepatobiliary system is the changes' determination in the clearance of various exogenous (including pharmacological) substances, which metabolism almost absolutely proceeds with the involvement of the hepatic cytochrome system [1], [2].

Currently, clearance tests are an important and integral component of assessing the functional state of the liver. They are a set of methods that allow you to determine the rate at which a certain substance is excreted from the body through the liver [3], [4].

Clearance tests in the context of the functional state of the liver are of great importance in the diagnosis and monitoring of diseases of this organ. They allow you to determine how effectively the liver performs its functions and help specialists choose the most optimal treatment methods [5].

The main goal of this study is to determine dose-dependent features of the kinetic curves of caffeine in the urine of laboratory animals.

Research methods and principles

The studies were carried out in the vivarium of the Department of Pharmacology and Toxicology of the FSBEI HE SPbSUVM. The studies were carried out in accordance with the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, the rules of Good Laboratory and Clinical Practice (GLP and GCP), as well as the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the

European Union dated 22 September 2010 on the Protection of Animals Used for Scientific Purposes [8], [9]. The study design was approved by the Bioethics Commission of the St. Petersburg State University of Veterinary Medicine.

Non-linear laboratory rats (18 males, 18 females, average body weight 180 g ± 4%, age 3 months) [6] purchased from the Federal State Unitary Enterprise "Laboratory Animal Nursery" "Rappolovo" were used for the study.

All animals were subjected to prophylactic quarantine (acclimatization period) before the study. During quarantine, each animal was examined daily (behavior and general condition), animals were observed in cages twice a day (morbidity and mortality) [7].

The following environmental conditions were maintained in the animal holding room: ambient temperature 18-24℃; relative humidity 50-60%; automatic change of 12-hour light period (06.00-18.00 – day, 18.00-06.00 – night); 100% ventilation without recirculation with air change of 7-12 room volumes per hour [8].

Rats were kept in polycarbonate cages on a 2150 cm^2 bedding. Sawdust of non-coniferous trees, sterilized in a dry heat oven, was used as bedding. For feeding the animals, LBK-120 complete feed for laboratory animals (Tosnensky feed mill), corresponding to state standard 34566-2019, was used. Filtered tap water was provided in standard autoclaved drinking bottles [8].

From the experimental animals, 3 groups were formed (6 females and 6 males each), each was injected subcutaneously with a 20% solution of caffeine sodium benzoate (Mosagrogen LLC, Russia) in increments of 50 mg/kg (50, 100, 150) calculated as pure caffeine. These dosages are justified by the fact that, according to most researchers, it is precisely this step, which falls within the therapeutic breadth of the drug, which gives the most predictable and detectable caffeine concentrations in blood plasma.

After 10 minutes after administration, electrocardiogram parameters were recorded in each animal using the Physiobelt wireless system for recording and analyzing animal ECG (Neurobiotics, Russia) in order to exclude animals from the experiment in the presence of negative reactions to the administered drug from the cardiovascular system.

After 1 hour, 4 hours and 8 hours, urine was taken from the animals using capillary tubes with an additional applicable urethral pressure [9], in which Strelova O.Yu. and Chuvina N.A. (2008) [10] recorded the level of caffeine. To implement the above technique, we used a UF-1100 model spectrophotometer (Shanghai Mapada Instruments Co., Ltd, China). The time intervals were chosen based on the registered half-life of the drug.

Mathematical and statistical processing of the obtained data was carried out using the Statistica 6.1 program. The arithmetic mean (M) and its mean error (m) were calculated. *The calculation of the significance of the difference (p) by Student's t-test was not carried out due to the exploratory nature of the study and the absence of comparison groups.*

Main results

The results of trace levels of caffeine in the urine of laboratory animals are presented in Table 1 and Figure 1.

Table 1 - Urinary caffeine levels after exogenous caffeine administration

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Administered dosage of the medicine (calculated as pure caffeine)	50 mg/kg		100 mg/kg		150 mg/kg	
T after injection / sex of animals	♂	♀	♂	♀	♂	♀
1 hour	0,825±0,17	0,846±0,12	2,415±0,39	2,487±0,51	4,45±0,79	4,512±0,84
4 hours	0,42±0,08	0,39±0,05	3,132±0,22	3,151±0,27	4,87±0,53	4,76±0,62
8 hours	0,205±0,03	0,175±0,07	2,61±0,19	2,57±0,11	4,061±0,31	4,23±0,27

At a dosage of 50 mg/kg, a standard linear decrease in blood levels of caffeine was observed with a sharp drop after 4 hours, which generally reflects the standard pharmacokinetics of this substance. Consequently, after 8 hours, the amount of caffeine in the body becomes barely noticeable, indicating a natural metabolism and normal liver function.

When using dosages of 100 and 150 mg/kg, a non-linear biotransformation of the substance, when the concentration reaches a maximum 4 hours after administration was observed. This is a standard pharmacokinetic indicator for high dosages that fall outside the therapeutic range of the drug and are associated with insufficient reactivity of the cytochrome P-450 enzyme system during the absorption of the substance. Compared to baseline and peak levels, serum caffeine at 8 hours can be considered "trace", which also indicates absence of metabolic changes.

As a result of the experiment, the initial and maximum levels of caffeine were determined in order to further study its changes during the artificial induction of various hepatopathies types in a particular experimental group of laboratory animals. These data provide an opportunity to study changes in caffeine levels during experimental hepatotoxicity in the same experimental animals. The non-linearity of the process of caffeine biotransformation at doses of 100 mg/kg and 150 mg/kg

indicates the need to use the above 3 dosage steps in subsequent experiments, in order the dynamics of caffeine biotransformation serve as a diagnostic criterion.

Conclusion

In the course of scientific research, it is planned to develop graphs called "caffeine" that reflect changes in the concentration of a substance in the organism depending on time and dosage. These graphs will help to identify the optimal dosage that is most relevant for assessing the functional state of the liver. Based on the elimination vector, it is possible to conclude about the influence on the pharmacokinetic characteristics of various drugs, expanding our understanding of the prognostic functions of changes in the clearance of these substances.

Thus, clearance tests are an integral part of a comprehensive evaluation of the functional state of the liver. They allow you to more accurately identify possible violations in the organism metabolism and take timely measures to maintain its health and functionality. With advances in scientific and medical technology, clearance tests are becoming more accurate and informative, contributing to the accurate diagnosis and effective treatment of liver disease.

Финансирование

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Конфликт интересов

Не указан.

Conflict of Interest

None declared.

Рецензия

Все статьи проходят рецензирование. Но рецензент или автор статьи предпочли не публиковать рецензию к этой статье в открытом доступе. Рецензия может быть предоставлена компетентным органам по запросу.

Review

All articles are peer-reviewed. But the reviewer or the author of the article chose not to publish a review of this article in the public domain. The review can be provided to the competent authorities upon request.

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