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CHANGES OF ANALGESIC EFFECT OF SODIUM DICLOFENAC UPON CONDITIONS OF OVERLOAD OF THE RATION WITH FAT AND EXPERIMENTAL DIABETES MELLITUS

Summary. *With the purpose of study of the influence of experimental diabetes mellitus and a high-fat diet on the analgesic effect of sodium diclofenac (substrate of cytochrome P4502C) the rats were fed by the ration with the increased content of fat for 4 weeks (50% of energy value of the ration against 20% in the control group) and severe diabetes mellitus was induced intraperitoneally by the injection of 70 mg/kg of Streptozotocin. At this the strengthening of the analgesic effect of sodium diclofenac because of the weakening of cytochromes - P4502C -dependent hydroxylation to inactive metabolites was observed. Such changes in pharmacodynamics should be counted upon when the necessity arises to prescribe medications - substrate CYP2C in case of obesity and diabetes mellitus.*
Key words: *sodium diclofenac, diabetes mellitus, high-fat diet, xenobiotics.*

Introduction

Diabetes mellitus, obesity and metabolic syndrome are the most widespread metabolic disturbances among the population of many countries, including Ukraine, and have already become a serious medical, social and economic problem [7]. These states are associated with severe pathology of the cardiovascular system, namely hypertensive and coronary diseases, cerebrovascular events, liver diseases, with the musculoskeletal system diseases and oncology diseases [11, 12]. The major cause of their occurrence is a common pathogenic factor that is insulin deficiency and (or) insulin resistance [14, 16]. Dysinsulinism or reduction of receptor sensitivity to insulin cause not only metabolism disorder of lipids, hydrocarbons, amino acids, but also cover almost all regulatory and metabolic systems of the body, including biotransformation of xenobiotics.

Metabolic changes in liver, caused by diabetes mellitus and obesity, may influence the processes of xenobiotics biotransformation, because metabolism of foreign substances proceeds in the most active way in this very organ. Diabetes mellitus and the overload of the ration with fat cause significant changes of the activity of enzymatic systems of oxidative and conjugation phases of metabolism of xenobiotics, their elimination, and therefore may induce changes in pharmacodynamics and, as a result, pharmacological effect on the body.

The *goal* of the research was the assessment of the influence of experimental diabetes mellitus and high-fat diet on the analgesic effect of diclofenac sodium (substrate of cytochrome P4502C).

Materials and methods

The research was conducted on white outbred male rats, with the weight of 145 - 185 g. The animals of the control group received semisynthetic ration, made up according to the recommendations of the literature [8]. The ration consisted of casein and maize starch in the ratio of 20 and 65% (200 and 650 g correspondingly). The ration also included 10% of fat (100 g), including 5% of pig fat (50 g) and 5% of sun flower oil (50 g). The diet also included 1% of the vitamins blend, cooked on starch (10 g), 3% of the salts blend (30 g) and 1% of cellulose (10 g). The blend of vitamins and salts was also a part of ration. The average caloric content of the above-mentioned ration in equivalent to 1 kg of animal feed made up 4740 kcal, when the protein content made up 1130 kcal (24%); carbohydrates - 2665 kcal/g (56%); lipids - 945 (20%). All the components were thoroughly mixed with 2,5 liters of water and were cooked under slow heating conditions. In all experiments animals' nutrition and water provision was ad libitum.

High-fat diet was developed on the basis of recommendations of literature [18]. Literature data testify that the ration with the content of fat of more than 50% (of dietary calories) causes liver diseases, which are accompanied by an increase in activity of alanine- and aspartate aminotransferase, and other liver diseases symptoms [13]. In the ration of this group of animals the quota of fat was increased to 50% of total calorie content by reducing the proportion of carbohydrates to 26%. The ration included 200 g of casein, 300 g of starch and 250 g of fat (125 g of hog-grease and 125 g of sun flower oil). Cellulose, the blend of vitamins and salts were added to the ration, as it is in the control group. As far as a total weight of such a ration was a bit less than that of the control ration (for 200 g), the volume of water while preparing the diet was increased from 2,5 l to 2,7 l. Thus, the control and laboratory diet were not only isocaloric (4740 kcal), but also contained the same amount

of proteins, vitamins and mineral salts. Before the use of a high-fat diet the animals were on the control diet for a week.

Another group of rats that had started receiving complete casein-starch ration a week before the experiments and had been receiving it throughout the experiment, was affected with severe experimental diabetes mellitus [3, 13]. Streptozotocin was injected one-time at a dose of 70 mg/kg intraperitoneally, using its solution in 0,1 M citrate buffer, pH 4,5. In the control group the animals received an equivalent volume of citrate buffer [13]. The animals became a part of the experiment in 2 weeks after the beginning of streptozotocin injection and with glucose level higher than 12 mmol/L [3]. The control group was comprised of intact rats (that did not not suffer from diabetes mellitus and were on a semi-synthetic diet). In additional control experiment nicotinamide at a dose of 230 mg/kg was injected intraperitoneally one-time 15 minutes before the injection of streptozotocin to prevent the effect of streptozotocin at a dose of 45 mg/kg. According to some sources this dose of nicotinamide almost completely negates the effect of streptozotocin [19].

Analgesic effect of diclofenac sodium was determined at the model of electric stimulation of the mucosa of the straight intestine of rats with a severe diabetes mellitus after injection at a dose of 10 mg/kg [1] and after 4 weeks of fat overload of ration.

The statistical analysis of the research results was done using biometry methods, the changes of indices were considered probable under $p<0,05$ [6].

Experiments on animals were carried out in compliance with national biotic standards [2].

Results. Discussion

Within 4 weeks of being on a high-fat diet the animals of experimental group were gaining weight somewhat faster, the content of blood glucose lowered along with a significant increase of the amount of free fatty acids and ketone bodies (table 1).

It has been found out that a high-fat diet consumption influences the pharmacological effect of diclofenac sodium (table 2).

Starting with the 4-th hour of the examination after injection of sodium diclofenac, its analgesic effect on animals that consumed a high-fat diet became apparently higher in 33%, than in rats that were on a ration with usual fat content. During the 6th hour of the experiment this difference reached 52,6%, and during the 8th hour - 56,9%, correspondingly.

Blood glucose level of animals who received 70 mg/kg of streptozotocin increased thrice, the increase of the level of ketone bodies sixfold indicated the severity of diabetes mellitus, the level of free fatty acids increased in 2,5 times (table 3).

We have found out that diabetes mellitus may cause changes of pharmacological effect of sodium diclofenac (substrate of cytochrome P4502C [5, 15, 17]) for rats (table 4). Starting from the 2nd hour of the observation the analgesic effect of diclofenac on rats that suffer from diabetes mellitus was 30% higher than for animals from the control group. In 4 hours the analgesic effect of sodium diclofenac of rats that suffer from diabetes mellitus increased in 67,4%, in 6 hours - in 93,2%, and in 8 hours - in 96,4%.

It is known that diclofenac after entering the organism becomes the object of intense metabolism. With the

Table 1. Indices of carbohydrate and lipid metabolism of rats that were on a high-fat diet (M±m and n=10).

Indices	Control	High-fat diet	p
Blood glucose, mmol/l	5,84±0,31	4,98±0,22	0,05
Free fatty acids, mmol/l	0,53±0,05	1,02±0,07	0,001
Ketone bodies, mmol/l	0,38±0,05	1,07±0,05	0,001

Table 2. The impact of a high-fat diet on the dynamics of analgesic effect of sodium diclofenac (10mg/kg perorally) for rats at the model of electric stimulation of the mucosa of the straight intestine (M±m; n=7- 8).

Duration of the experiment, hours	Changes of pain sensitivity threshold, in % to original values	
	Control, n=7	A high-fat diet, n = 8
0,5	9,91±0,73	12,1±1,78
1	17,6±1,46	21,7±2,26
2	22,7±1,33	27,0±1,71
4	23,3±1,75	31,0±1,98*
6	19,2±1,22	29,3±2,15*
8	13,7±1,08	21,5±1,66*

Note: 1. * - differences ($p<0,05$) of average values compared to control are probable; 2. Pain sensitivity threshold before injection of diclofenac is taken as 100%.

Table 3. Indices of carbohydrate and lipid metabolism of rats that suffer from diabetes mellitus (M±m).

Indices	Control group, n=11	Streptozotocin 70 mg/kg, n=12	Nicotinamide + streptozotocin, n=10
Blood glucose, mmol/l	6,02±0,28	18,0±1,06*	6,90±0,36
Free fatty acids of blood serum, mmol/l	0,62±0,06	1,58±0,11*	0,77±0,04
Ketone blood bodies (by maleic hydrazide), mmol/l	0,40±0,05	2,40±0,23*	0,53±0,05

Note: * -differences ($p<0,05$) regarding the control group are probable.

Table 4. The impact of diabetes mellitus on the dynamics of analgesic effect of sodium diclofenac of rats (M±m; n=7-8).

Duration of the experiment, hours	Control, n = 7	Diabetes mellitus, n = 8
0,5	9,91±0,73	12,4±1,30
1	17,6±1,46	22,9±2,32
2	22,7±1,33	29,5±2,53*
4	23,3±1,75	39,0±2,38*
6	19,2±1,22	37,1±2,40*
8	13,7±1,08	26,9±1,92*

Note: * - differences ($p<0,05$) of average values compared to control are probable.

participation of cytochromes P4502C7, 2C9, 2C11 diclofenac is being hydroxylated, mainly to 4'- Hydroxy Diclofenac, which is further succumbs to conjugation with glucuronic acid and sulfate [9, 15]. Therewith diclofenac metabolites are almost devoid of pharmacological activity, that is why analgetic activity of the product is practically determined by the concentration of its unaltered form, therefore the impact on the speed of biotransformation of sodium diclofenac will significantly influence its therapeutic properties.

We have found out that diabetes mellitus and the overload of the ration with fat cause significant quantitative and qualitative changes of metabolic system of xenobiotics in rats bodies, which manifest themselves at all levels of organization of this system: both at the subcellular level (activity of xenobiotic-metabolizing enzymes of the first and second phases of metabolism in subcellular fractions of liver, kidneys and lungs) and at the level of the whole body (elimination of model xenobiotics and their metabolites with excretion, pharmacological effect and toxicity) [4, 9]. The main pathogenetic factors that occur in the process of overload of the body with fat and the diabetes mellitus development, with which the changes in activity of enzymes of metabolism of xenobiotics are associated to the fullest extent, are first of all hyperketonemia, the increase of free fatty acid concentration, the degree of hepatic steatosis (accumulation of triglycerids in the liver) and activation of the gluconeogenesis processes (which were judged upon on the basis of the increased glucose-6-phosphatase activity). The changes in the activities of enzymes of xenobiotics biotransformation at these states have systemic character and manifest themselves as violations of the processes of elimination of the last at the level of the whole body and as changes of body reaction on their activities.

Diabetes mellitus causes the increase of pharmacological activity of sodium diclofenac because of the decrease of the activity of substrate of cytochrome P4502C [4], which is the main metabolizer of nonsteroidal anti-inflammatory drugs, including diclofenac [5, 15, 17]. Whereas only the unaltered form of diclofenac has pharmacological activity and the products of its hydroxylation are devoid of analgesic action, then the weakening of cytochromes - P4502C-dependent hydroxylation to inactive metabolites at diabetes mellitus causes the increase of concentration of pharmacologically active form of the medication and its pharmacological action.

We have previously shown that the overload of the ration of rats with fats causes the inhibition of the activity of cytochrome P4502C in liver microsomes [4, 9]. Thus, we may assume that the increase of the pharmacological activity of sodium diclofenac at the final terms of the pharmacodynamic curve is the result of the inhibition of its elimination from the body of the animals that were on a high-fat diet.

The received data allows us to draw a conclusion about the slowdown of elimination of pharmacologically active unaltered form of sodium diclofenac from the rats' bodies that suffer from diabetes mellitus and the overload of the ration with fat apparently because of the reduced activity of cytochrome P4502C, found by us (Indomethacin-O-demethylase and hexobarbital hydroxylase) [4, 9]. This explains the increase of analgesic effect of diclofenac at these pathological conditions.

Conclusions and prospects of further developments

1. Diabetes mellitus and the overload with fats strengthen the analgesic effect of sodium diclofenac. Such a significant increase of the pharmacological activity of sodium diclofenac in the final terms of the pharmacodynamic curve is the result of the inhibition of its elimination from the bodies of the animals that suffered from diabetes mellitus and the overload of the ration with fats that occurs because of the reduction of the activity of cytochrome P4502C which catalyses the transformation of sodium diclofenac in pharmacologically inactive hydroxylated metabolites.

2. In case of prescription of sodium diclofenac and other medicinal agents - substrates of cytochrome P4502C to the patients who suffer from diabetes mellitus, obesity, hepatic steatosis, the correction of their dose is necessary.

Perspective are researches of other effects of sodium diclofenac, as model xenobiotics among nonsteroidal anti-inflammatory drugs, and other medicinal agents - substrates of cytochrome P4502C in the conditions of pathometabolism of fats and carbohydrates. What will give an opportunity to increase their efficiency and safety at the states, that is accompanied by ketoacydosis, lipidemia.

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ЗМІНИ АНАЛЬГЕТИЧНОГО ЕФЕКТУ ДИКЛОФЕНАКУ НАТРІЮ ЗА УМОВ ПЕРЕВАНТАЖЕННЯ РАЦІОНУ ЖИРАМИ ТА ЕКСПЕРИМЕНТАЛЬНОГО ЦУКРОВОГО ДІАБЕТУ

Резюме. *З метою вивчення впливу експериментального цукрового діабету та високо жирової дієти на аналгетичний ефект диклофенаку натрію (субстрату цитохрому Р4502С) щурі протягом 4-х тижнів перебували на раціоні з підвищеним вмістом жирів (50% енергетичної цінності раціону проти 20% в контролі), а важкий цукровий діабет був індукований внутрішньоочеревинним введенням 70 мг/кг стрептозотоцину. В цих умовах аналгетичний ефект диклофенаку натрію посилюється внаслідок сповільнення його цитохром Р4502С залежного гідроксилювання до неактивних метаболітів. Такі зміни фармакодинаміки слід враховувати при потребі призначення лікарських засобів субстратів CYP2C при ожирінні та цукровому діабеті.*

Ключові слова: *цукровий діабет, високожирова дієта, ксенобіотики, диклофенак натрію.*

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ИЗМЕНЕНИЯ АНАЛЬГЕТИЧЕСКОГО ЭФЕКТА ДИКЛОФЕНАКА НАТРИЯ В УСЛОВИЯХ ИЗБЫТОЧНОЙ НАГРУЗКИ РАЦИОНА ЖИРАМИ И ЭКСПЕРИМЕНТАЛЬНОГО САХАРНОГО ДИАБЕТА

Резюме. *С целью изучения влияния экспериментального сахарного диабета и высокожировой диеты на анальгетический эффект диклофенака натрия (субстрата цитохрома Р4502С) крысы в течении 4-х недель получали рацион с повышенным содержанием жиров (50% энергетической ценности рациона против 20% в контроле), тяжелый сахарный диабет был индуцирован внутрибрюшинным введением 70 мг/кг стрептозотоцина. В этих условиях анальгетический эффект диклофенака натрия усиливается вследствие замедления его цитохром Р4502С зависимого гидроксилирования до неактивных метаболитов. Такие изменения фармакодинамики следует учитывать при необходимости назначения лекарственных средств субстратов CYP2C пациентам с ожирением и сахарным диабетом.*

Ключевые слова: *сахарный диабет, высокожировая диета, ксенобиотики, диклофенак натрия.*

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ІНТРАМУРАЛЬНІ АРТЕРІЇ МІОКАРДА ПРИ ХРОНІЧНІЙ ІШЕМІЧНІЙ ХВОРОБІ СЕРЦЯ

Резюме. *У хворих на хронічну ІХС вивчали морфофункціональний стан інтрамуральних артерій серця. Кардіобіопсії лівого шлуночка отримували з басейну стенозованої магістральної судини та з регіонів, розташованих поза ним. Встановлено*