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COMPARATIVE PHARMACOKINETIC INVESTIGATION OF KETOROLAC AFTER INTRANASAL AND INTRAMUSCULAR ADMINISTRATION IN RABBITS

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Key words: ketorolac; nasal spray; pharmacokinetics

In order to create the original Ketorolac nasal spray the comparative pharmacokinetic study of ketorolac has been conducted. The investigation has been performed using a single intranasal or intramuscular administration of ketorolac in rabbits. The HPLC-method, including pre-solid phase extraction of the active substance from plasma and the vacuum concentration of the sample, have been used for quantitative determination of ketorolac in the animal plasma. The main pharmacokinetic constants of ketorolac for intranasal and intramuscular administration in rabbits have been calculated. The time of achieving the peak concentration for both ways of administration was 0.5 hours, the maximal concentration of ketorolac administrated intranasally was 3298.5 ng/ml, intramuscularly – 7337.5 ng/ml. However, in case of intranasal administration of ketorolac, unlike its intramuscular injection, within 0.5-1.5 hours after peak-reaching the plasma concentration of ketorolac declined slightly (10%); it was the evidence of longer absorption of ketorolac from the administration site. The blood circulation time of ketorolac provided by intranasal administration was 4.4 h and it lasted longer comparing to intramuscular injection – 2.5 h. In general, intranasal administration of ketorolac provides a high relative bioavailability (~71-86%) and can be considered as an alternative regimen in treating acute pain.

Nowadays the nomenclature of systemic medicines that are administrated intranasally is constantly extending. The intranasal way of drug delivery is gaining a value for systemic therapy due to the good absorptive properties of the nasal mucus that provides a comparatively high plasma level of active ingredients.

The important stage of their pharmaceutical development, especially by keeping indications for medical use, is the assessment of bioavailability and other pharmacokinetic parameters. It allows to determine an optimal active ingredient content and general formulation for providing the required therapeutic plasma concentration of the active ingredient.

The effectiveness of intranasal use of ketorolac being a non-steroidal anti-inflammatory drug widely used for relief of acute pain has been proven by a number of resent investigations [8, 9, 11, 12]. Nowadays ketorolac is used as 10 mg tablets and 3% solution for injections introduced deeply intramuscularly [1, 6, 10]. For oral use the maximal daily dose of ketorolac is 40 mg, whereas the therapy duration should not exceed 5 days. The parenteral administration of ketorolac makes it possible to increase the daily dose to 90 mg by 3 injections per day; the therapy duration is not more than 2 days [1, 6, 10]. However, for certain patients the oral intake of ketorolac is unfavourable because of its ulcerogenic effect, whereas the repetitive injections cause additional discomfort for a patient, increase the risk of local side effects and are labour-consuming for the medical staff [11].

The above-mentioned facts stipulate the expediency of developing the original ketorolac dosage form – a nasal spray; it would allow to increase the permitted course of treatment and at the same time to avoid the unwanted oral or injectable administration, as well as to facilitate the "on demand" use of an analgesic in patient-controlled analgesic regimens [4, 11]. On this background the pharmaceutical developing of Ketorolac nasal spray has been performed. The research was perfomed by the State Scientific Centre of Drugs and Medical Products for "Moschimpharmpreparaty" named after N.A. Semashko JSC (Russia) under the supervision of prof. Lyapunov M.O.

The aim of the research was the comparative investigation of bioavailability and other pharmacokinetic characteristics of ketorolac after intranasal and intramuscular administration in rabbits.

Materials and Methods

Twelve conscious puberal rabbits (chinchilla breed) of both genders weighing 2.7 to 4.9 kg (the average weigh is 3.9 kg) were used in the study. Before the experimental period all animals were kept under the standard vivarium conditions. The animals did not receive any medicines during 3-day period before the experiment. The investigation was performed according to the "Regulations for the animal use in biomedical investigations" [3] and requirements of the State Expert Centre of the Ministry of Public Health of Ukraine [2].

The animals were taken either a single intranasal (i.n.) ketorolac spraying in the dose of 2 mg/kg or

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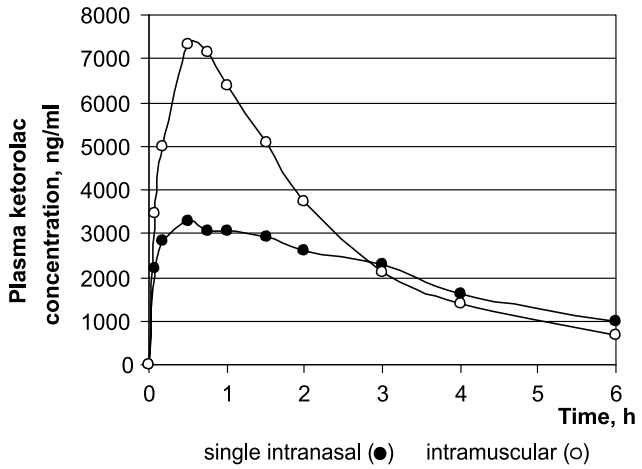

Fig. Dynamics of the ketorolac concentration in the blood plasma after a single intranasal and intramuscular administration in rabbits in the dose of 2 mg/kg

a single intramuscular (i.m.) injection of ketorolac of 30 mg/ml solution at the same dose. Blood samples for the analysis were taken from the marginal ear vein and were collected to the heparinized vials before and in 5, 10, 30, 45, 60, 90, 120, 180, 240 and 360 min after drug administration. Blood samples were centrifuged at 3000 rpm for 5 min. The plasma samples obtained were stored at –22°C before carrying out analytical procedures for the ketorolac content.

Blood concentrations of ketorolac have been determined by the HPLC method with pre-solid phase extraction of the active substance from the animal plasma and the vacuum concentration of the sample developed in our laboratory.

During sample preparation unfreeze the plasma samples at the room temperature. To 0.7 ml of the plasma sample add 50 ml of 20% phosphoric acid solution, then centrifuge at 6000 rpm for 7 min at the room temperature, after that collect 1 ml of the supernatant fluid and transfer into a cartridge for solid-phase extraction (Oasis – HLB; Waters).

Wash out the endogenous plasma substances from the cartridge twice with 1 ml of water and 1 ml of 5% methanol, then pass 1 ml of methanol through the cartridge. Place the test-tubes with the eluate collected into the vacuum rotating concentrator, and evaporate the eluate to dryness at 60°C. Dissolve the dry residue in 250 ml of the mobile phase.

The HPLC-system "Perkin Elmer" Series 200 (USA) was used for the analysis. The chromatographic conditions were as follows: the chromatographic column – ZORBAX SB-C18; 250 × 4.6 mm, 5 mm; the precolumn – ZORBAX SB-C18; 250 × 4.6 mm, 5 mm; the mobile phase – 1.3 mM ortophosphoric acid (component A) and acetonitril (component B) in the ratio of 57 : 43 (v/v); the detection wavelength – 301 nm; the column thermostat temperature – 35°C; the autosampler thermostat temperature – 16°C; the eluate fluid rate – 1.0 ml/min; the injection volume – 50 ml. The retention time of ketorolac was 5.5÷7.5 min, the cycle time was 1 min.

The concentrations of ketorolac in plasma samples were calculated using the calibration curve of the "peak area – concentration" dependence obtained by the method of least squares with the weighting factor = 1/x. The equation of the calibration curve was: $y = a \times x + b$ where y – is the ketorolac peak square (S_{KET}); x – is the ketorolac concentration (ng/ml); a = 311.2745; b = –347.711; the regression coefficient r = 0.99948.

Under the above-mentioned conditions of the sample preparation and chromatographic analysis the calibration curve of ketorolac was linear within the concentration range from 40 to 16000 ng/ml; the lowest detection limit of the method was 40 ng/ml. In general, the results of the validation research indicate that the given method of quantitative determination of ketorolac in plasma meets the acceptance criteria for bioanalytical method [7] in parameters of selectivity, response function, precision, accuracy, extraction degree, and it can be used for pharmacokinetic studies of ketorolac dosage forms.

The peak concentration (C_{max}) was determined as the highest measured value of every rabbit with the relevant time to peak concentration (T_{max}). Other parameters were calculated by the model-independent method using WinNonLin (Pharsight Corp., USA) programme. The statistical processing of data was performed using MicroSoft® Office Excel 2003 SP2 (MicroSoft Corporation, USA) programme.

Results and Discussion

Dynamics of the ketorolac concentration in the blood plasma of rabbits observed after i.n. and i.m. administration are shown in Fig. Ketorolac after i.m. administration is well absorbed. The ketorolac concentration increases rapidly reaching the value of 3468.7 ng/ml (50% of C_{max}) in 5 min after injection and 4990 ng/ml (70% of C_{max}) in 10 min. The absolute ketorolac concentrations after i.n. administration are slightly lower, but in terms of percentage as for C_{max} they exceed those after i.m. injection comprising 2221.6 ng/ml (67% of C_{max}) in 5 min, 2850 ng/ml (86% of C_{max}) in 10 min after administration. The time of achieving the peak concentration (T_{max}) of ketorolac after both ways of administration was 0.5 h (Table).

The absolute C_{max} value of ketorolac after i.m. administration is 7337.46 ng/ml; it is 2.2 times higher than after i.n. instillation – 3298.53 ng/ml. The absolute ke-

Table

The main pharmacokinetic parameters of ketorolac with a single intranasal and intramuscular administration in rabbits

Pharmacokinetic parameters	The way of administration	
	intranasally	intramuscularly
C_{max}, ng/ml	3298.53	7337.46
T_{max}, h	0.5	0.5
CL_T, ml/h	117.54	101.33
K_{el}, h^{-1}	0.239	0.374
$T_{1/2}$, h	2.91	1.86
MRT, h	4.36	2.50
V_z, ml/kg	490.88	271.38
$AUC_{0\to t}$, ng×h/ml	12784.15	17902.95
$AUC_{0\to\infty}$, ng×h/ml	17015.84	19737.55
$AUC_{0\to t}/AUC_{0\to\infty}$, %	90.70	75.13
$C_{max}/AUC_{0\to t}$, h^{-1}	0.258	0.410
f', %	71.41	100.00
f'', %	86.21	100.00

torolac concentrations after i.m. injection significantly exceed those after i.n. instillation within the period from 0.5 to 1 h after administration. With i.m. administration of ketorolac after reaching C_{max} a rather rapid decline of the ketorolac concentration in plasma is observed. The plasma ketorolac level decreases more than in 3 times up to 3 h after administration, while in 6 h the ketorolac plasma level is less than 10% of C_{max}.

When ketorolac is administred i.n., unlike i.m. injection, the pharmacokinetic curve does not show a sharp peak, the maximum has a plateau shape. Within 0.5-1.5 h after achieving C_{max} the ketorolac concentration in plasma decreases only in 10%; it indicates the longer systemic absorption of ketorolac from the nasal mucus comparing to i.m. administration. Then a gradual decline of the ketorolac concentration in the blood plasma is observed: up to 70% of C_{max} in 3 h, up to 31% of C_{max} in 6 h after administration.

The main pharmacokinetic parameters of ketorolac after i.n. and i.m. administration in rabbits are shown in Table. The parameter value characterizing the ketorolac absorption rate ($C_{max}/AUC_{0\to t}$) differs for i.n. and i.m. administration (0.258 h^{-1} and 0.410 h^{-1}, respectively), and it indicates the longer absorption of ketorolac from the nasal cavity. Such peculiarity of absorption in i.n. administration provides 1.75 times greater ketorolac circulation than in i.m. injection, while the mean retention time (MRT) of ketorolac is 4.36 h for i.n. and 2.50 h for i.m. administration. The values of the apparent volume of distribution (V_z) for both ways of administration (490.88 ml/kg i 271.38 ml/kg, respectively) give the evidence of the absence of ketorolac deposition by tissues. The elimination of ketorolac is slower after i.n. administraton than after i.m. injection; it is reflected in the elimination rate constant (K_{el}) – 0.239 h^{-1} and 0.374 h^{-1}, respectively, and the elimination half-life time ($T_{1/2}$) – 2.91 h and 1.86 h, respectively.

In general, i.n. administration of ketorolac provides a high relative bioavailability f' – 71.41% and f'' – 86.21% in comparison with i.m. administration; it corresponds to the published data about similarity of pharmacokinetic parameters of ketorolac after i.n. and i.m. administration [5].

CONCLUSIONS

1. The method of quantitative determination of ketorolac in the blood plasma, which includes HPLC with pre-solid phase extraction of the active ingredient from the plasma and vacuum concentration of the sample, has been developed. The method proposed meets the acceptance criteria for bioanalytical methods and can be used for pharmacokinetic studies of ketorolac dosage forms.

2. Intranasal administration of ketorolac provides slower and sustained absorption, and the greater blood circulation time in comparison with i.m. administration.

3. Intranasal administration of ketorolac provides a high relative bioavailability (~71-86%) and can be considered as a viable alternative formulation in treating acute pain.

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ПОРІВНЯЛЬНЕ ДОСЛІДЖЕННЯ ФАРМАКОКІНЕТИКИ КЕТОРОЛАКУ ПРИ ІНТРАНАЗАЛЬНОМУ ТА ВНУТРІШНЬОМ'ЯЗОВОМУ ВВЕДЕННІ КРОЛИКАМ

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Ключові слова: кеторолак; спрей назальний; фармакокінетика

З метою створення оригінального препарату Кеторолак спрей назальний проведене порівняльне дослідження фармакокінетики кеторолаку при його одноразовому інтраназальному та внутрішньом'язовому введенні кроликам. Для кількісного визначення кеторолаку в плазмі крові тварин використовували метод ВЕРХ з попередньою твердофазною екстракцією діючої речовини з плазми і концентруванням проби під вакуумом. Розраховані основні фармакокінетичні константи кеторолаку при інтраназальному і внутрішньом'язовому введенні кроликам. Час досягнення максимальної концентрації кеторолаку при обох шляхах введення склав 0,5 год, максимальна концентрація препарату при інтраназальному введенні становила 3298,5 нг/мл, при внутрішньом'язовому – 7337,5 нг/мл. Проте, за інтраназального введення кеторолаку, на відміну від ін'єкційного, впродовж 0,5-1,5 годин після досягнення максимуму концентрація кеторолаку в плазмі знижується лише на 10%, що свідчить про більш тривалу абсорбцію препарату з місця введення. Інтраназальне введення кеторолаку забезпечує більш тривалий час циркуляції в крові порівняно із внутрішньом'язовим введенням, що склало, відповідно 4,4 та 2,5 год. Загалом, при інтраназальному введенні кеторолак характеризується високим ступенем відносної біодоступності (~71-86%) та може розглядатися як альтернатива ін'єкційному введенню у схемах лікування виразного больового синдрому.

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ФАРМАКОКИНЕТИКИ КЕТОРОЛАКА ПРИ ИНТРАНАЗАЛЬНОМ И ВНУТРИМЫШЕЧНОМ ВВЕДЕНИИ КРОЛИКАМ

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Ключевые слова: кеторолак; спрей назальный; фармакокинетика

С целью создания оригинального препарата Кеторолак спрей назальный проведено сравнительное исследование фармакокинетики кеторолака при его одноразовом интраназальном и внутримышечном введении кроликам. Для количественного определения кеторолака в плазме крови животных использовали метод ВЭЖХ с предварительной твердофазной экстракцией действующего вещества из плазмы и концентрированием пробы под вакуумом. Рассчитаны основные фармакокинетические константы кеторолака при интраназальном и внутримышечном введении кроликам. Время достижения максимальной концентрации кеторолака при обоих путях введения составляет 0,5 ч, максимальная концентрация препарата при интраназальном введении составила 3298,5 нг/мл, при внутримышечном – 7337,5 нг/мл. Однако, при интраназальном введении кеторолака, в отличие от инъекционного, в течение 0,5-1,5 ч после достижения максимума концентрация кеторолака в плазме снижается лишь на 10%, что свидетельствует о более продолжительной абсорбции препарата из места введения. Интраназальное введение кеторолака обеспечивает более длительное время циркуляции в крови сравнительно с внутримышечным введением, которое составило, соответственно, 4,4 и 2,5 ч. В целом при интраназальном введении кеторолак характеризуется высокой степенью относительной биодоступности (~71-86%) и может рассматриваться как альтернатива инъекционному введению в схемах лечения выраженного болевого синдрома.