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A COMPARATIVE STUDY OF THE ANALGESIC AND ANTIPYRETIC EFFECT OF THE INTERLEUKIN-1 RECEPTORS RECOMBINANT ANTAGONIST

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Key words: raleukin; acetic acid writhings; milk fever; antipyretic; analgesic effect

In recent years the special attention is focused on the problem of creation of effective and safe anti-inflammatory drugs and their introduction into medical practice. According to the modern concepts the activation of cytokines is one of the inflammatory process triggers. Thus, correction of quantitative, qualitative and functional disorders of cytokine regulation, in particular by blocking the receptors that are sensitive to cytokines, such as interleukin-1 (IL-1), is one of the promising areas of the modern anti-inflammatory therapy. The article presents the results of the comparative experimental study of the analgesic and antipyretic properties of raleukin IL-1 receptors recombinant antagonist. It has been found that on the model of acetic acid writhings in mice raleukin exhibits a moderate analgesic activity, which is considerably inferior to analgin. However, it should be noted that raleukin – has shown the analgesic effect in the dose of 3 mg/kg, which is 17 times less than the dose of the reference drug. On the model of milk fever in rats raleukin has shown the expressed antipyretic effect in the preventive mode of introduction and the moderate one in the therapeutic mode. The difference in the intensity of the antipyretic activity of the drug in the various modes of introduction can probably be explained by the fact that during the therapeutic mode we deal with the consequences of the IL-1 propyrogenic effect as the result of the cytokine cascade launch; therefore, IL-1 receptor blocking in this case does not affect the intensity of the current process. And the preventive introduction of raleukin allows to delay the launch of the cytokine cascade. The pharmacological properties of raleukin determined allow to consider this drug to be a promising anti-inflammatory agent. It is reasonable to conduct further a profound experimental study with the aim to determine the peculiarities of its therapeutic effect.

It is known that despite the success achieved in the treatment of inflammatory diseases their pharmacotherapy is still an acute problem of the contemporary medicine [5, 6].

Non-steroid anti-inflammatory drugs (NSAIDs) are one of the drug groups widely used for treating inflammations. However, despite their undoubted clinical efficacy NSAIDs exhibit a number of serious side effects greatly limitting their use in clinics [1, 9]. Typical side effects of NSAIDs are associated with the mechanism of action of this group of drugs, and avoiding them is almost impossible [9, 13]. Therefore, in recent years a special attention is drawn to the problem of creation of effective and safe anti-inflammatory drugs, which would have the mechanism of action differing from the traditional anticyclooxygenase one with the impact on all stages of development of the inflammatory process and would be deprived of the most common complications of modern NSAIDs, as well as their introduction into medical practice [6, 10].

According to the modern concepts the activation of cytokines is one of the inflammatory process triggers. Cytokines promotes the release of lysosomal enzymes and biologically active substances, which exhibit the prooxidant action, and contribute to the dissociation processes of oxidative phosphorylation and tissue respiration, resulting in imbalance of the cellular metabolism and destruction of cells [6, 8, 10].

Cytokines also intensify the arachidonic acid conversion cascade and the synthesis of eicosanoids, which are mediators of inflammation, and in their turn, contribute to the further formation of cytokines and free radicals [4, 7, 12]. Hence, there is a vicious circle, which can be broken with the help of drugs with the major effect of inhibition of cytokines, particularly interleukin-1 (IL-1). Therefore, correction of quantitative, qualitative and functional disorders of cytokine regulation, in particular by blocking the receptors that are sensitive to cytokines, such as interleukin-1 (IL-1), is one of the promising areas of the modern anti-inflammatory therapy [3, 4, 8, 11, 15].

Considering the fact that one of the important characteristics of IL-1 is pyrogenicity and taking into account the certain role of IL-1 in formation of the pain reaction [6, 11] the aim of the work was to conduct a comparative experimental study of analgesic and antipyretic properties of the original recombinant receptor antagonist IL-1 raleukin obtained at the St. Petersburg State Research Institute of Highly Pure Bioprepararations (Russian Federation).

Materials and Methods

The analgesic activity of raleukin was studied on the model of acetic acid writhings in mice. The

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Table 1

The study of the analgesic activity of raleukin on the model of acetic writhings in mice (n=6)

Conditions of the experiment	Dose, mg/kg	Number of writhings for 20 min of the experiment	Analgesic activity, %
Control pathology	–	19.7±1.6	–
Raleukin	3	15.1±1.2*/**	23.5
Analgin	50	8.6±1.4*	56.3

Notes:
1) * – deviation of the index is significant in relation to the control pathology p≤0.05;
2) ** – deviation of the index is significant in relation to analgin p≤0.05;
3) n – the number of animals in the group.

reference drug was metamizole sodium (analgin). It is recommended by the State Expert Centre of MoH of Ukraine as a reference drug for study the analgesic activity of potential non-narcotic analgesics [2].

The experimental animals were divided into three groups: the first group was the control mice treated with 0.7% solution of acetic acid introduced intraperitoneally in the amount of 0.1 ml per 10 g of the body weight; the second group of animals received raleukin subcutaneously 30 min prior to the introduction of algogen in the dose of 3 mg/kg determined in the previous studies; the third group of mice received analgin intramuscularly 30 min prior to the introduction of algogen in the dose of 50 mg/kg. This dose is ED_{50} by the analgesic effect [2, 5].

When introducing the solution of acetic acid the animals had "writhings" – spasmodic contractions of abdominal muscles accompanied with stretching of hind limbs and back arching [2]. The number of writhings was counted for 20 min. The analgesic activity of the substances studied was determined by the difference in the number of writhings in the experimental and control groups.

The antipyretic properties of raleukin were determined on the model of milk fever in rats. The reference drug was diclofenac sodium. It is a classic anti-inflammatory drug with a marked antipyretic activity [2].

The peculiarities of the antipyretic action of the drugs under research were determined in the preventive and therapeutic mode. Laboratory animals were divided into the following groups (5 rats each): the first – the group of the control pathology, the animals of the second group were introduced subcutaneously a single dose of 3 mg/kg of raleukin, the animals of the third group were introduced intramuscularly diclofenac sodium in the dose of 8 mg/kg [2, 5].

In the preventive mode raleukin and the reference drug were introduced 1 hour prior to the introduction of a pyrogen. In the therapeutic mode the drugs under research were introduced at the fever maximum, i.e. in 2 hours after introduction of a pyrogenic substance.

The body temperature of animals was measured by entering a TSM-2 thermometer into the rectum with the electrode depth of 0.5 cm q.h. for 5 hours after the drug introduction. The antipyretic activity was determined by the difference in the body temperature of rats of the experimental group and the control pathology group and was expressed in percentage.

The study was performed on 30 mature white male rats weighing 180-260 g and 18 white male mice weighing 15-24 g grown in the breeding nursery of the vivarium of the Central Research Laboratory at the National University of Pharmacy certified by the State Expert Centre of MoH of Ukraine. The work with animals was carried out under "The ethics of research involving animals" approved by the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" and the resolution of the First National Congress on Bioethics.

In the case of accounting results in the form of mean ± standard error the statistical reliability of intergroup differences was calculated by Student t-test with Bonferroni correction.

Results and Discussion

The results are given in Table 1.

The number of writhings in the control pathology group of animals after introduction of acetic acid solution was 19.7±1.6. On the background of raleukin the number of writhings significantly reduced by 1.3 times compared to the control pathology group and was 15.1±1.2. When using analgin the number of writhings reduced by 2.3 times to 8.6±1.4, being significantly less than in mice treated with raleukin.

As shown by the results of the experiment, the analgesic effect of raleukin was equal to 23.5%. However, the drug under research was about twice worse than analgin with the analgesic activity of 56.3%.

When studying the antipyretic properties of raleukin in the preventive mode of introduction one hour after introduction of a pyrogenic substance the increase of the body temperature by 0.8-1.2°C was registered in all groups of the experimental animals (1.2°C – in the control pathology group of animals, 0.8°C – in the groups of animals received the drugs under research).

After the second hour the further increase of the animals' body temperature was observed: in the control group of animals – by 1.8°C; in the group of animals received raleukin – by 1.2°C; in the group of animals received diclofenac so-

Table 2

Dynamics of the antipyretic activity of raleukin in the preventive mode of introduction (n=5)

Experimental conditions	Control pathology		Raleukin, 3 mg/kg		Diclofenac sodium, 8 mg/kg	
	T°C	ΔT	T°C	ΔT	T°C	ΔT
Initial background	38.1±0.4	-	38.2±0.5	-	38.1±0.3	-
1st hour	39.3±0.6**	1.2	39.0±0.4**	0.8	38.9±0.5**	0.8
2nd hour	39.9±0.5*/**	1.8	39.4±0.5	1.2	39.7±0.2*/**	1.6
3rd hour	41.2±0.3*	3.1	39.9±0.3*	1.7	41.0±0.5*	2.9
4th hour	41.5±0.4*	3.4	40.4±0.2*/•	2.2	41.3±0.3*	3.2
5th hour	40.9±0.2*	0.6	39.0±0.4**	1.4	40.1±0.4*/**	1.2
Antipyretic effect, %	-		63.6		37.5	

Notes:
1) * – deviation of the index is significant in relation to the initial temperature (the initial background), $p \le 0.05$;
2) ** – deviation of the index is significant in relation to the temperature at the fever maximum (the 4th hour), $p \le 0.05$;
3) • – deviation of the index is significant in relation to diclofenac sodium, $p \le 0.05$;
4) n – the number of animals in the group.

dium – by 1.6°C. In three hours the increase of temperature by 3.1°C was registered in the control pathology group of animals. The groups of animals preventively receiving raleukin and diclofenac sodium also experienced an increase of the body temperature, but the temperature was significantly lower than in the control animals by 1.7°C and 2.1°C, respectively. Thus, introduction of raleukin helps to reduce the body temperature of animals at the fever maximum by 1.4°C, and introduction of diclofenac sodium – by 1°C compared to the same index in the control pathology group.

According to the data of Table 2 the fever maximum in all three groups of the experimental animals was observed at the end of the fourth hour after introduction of a pyrogen. The temperature of animals in the groups treated with the drugs under research was significantly different from the body temperature of the control animals by 2°C and 2.2°C, respectively.

At the end of the study (in five hours) the temperature of all experimental rats decreased by 0.6-1.4°C compared to the fever maximum temperature, but the temperature of the control pathology group and animals treated with diclofenac sodium significantly differed from the initial background. The temperature of animals treated with raleukin was 39°C, and it was significantly less than the temperature of the control pathology group. The average temperature of the animals received diclofenac sodium was higher than that of the previous group (40.1°C) and significantly differed from the initial background.

Thus, the average antipyretic activity of raleukin in the preventive mode (63.6%) exceeded 1.7 times the same index of diclofenac sodium (37.5%).

In the therapeutic mode of introduction the fever maximum was observed in three hours after the pyrogen introduction. All three groups of the experimental animals had a significant increase of the body temperature by 2.3-3.1°C. After the fourth hour the body temperature of the experimental animals in all groups started to decrease, but the significant changes in temperature compared to the temperature of the fever maximum were observed in the group of animals recei-

Table 3

Dynamics of the antipyretic activity of raleukin in the therapeutic mode of introduction (n=5)

Experimental conditions	Control pathology		Raleukin, 3 mg/kg		Diclofenac sodium, 8 mg/kg	
	T°C	ΔT	T°C	ΔT	T°C	ΔT
Initial background	38.6±0.3	-	38.8±0.5	-	38.8±0.4	-
3rd hour	41.7±0.2*	3.1	41.2±0.3*	2.4	41.1±0.5*	2.3
4th hour	41.5±0.4*	0.2	40.9±0.2*	0.3	40.3±0.3*	0.8
5th hour	41.3±0.3*	0.4	40.5±0.1*/•	0.7	39.3±0.4**	1.8
Antipyretic effect, %	-		29.2		78.3	

Notes:
1) * – deviation of the index is significant in relation to the initial temperature (the initial background), $p \le 0.05$;
2) ** – deviation of the index is significant in relation to the temperature at the fever maximum (the 3rd hour), $p \le 0.05$;
3) • – deviation of the index is significant in relation to diclofenac sodium, $p \le 0.05$;
4) n – the number of animals in the group.

ved diclofenac sodium. The control pathology group and rats received raleukin had only a tendency to the temperature reduction (Table 3).

In five hours the significant temperature reduction was registered in both groups received the drugs under research, but the temperature in the diclofenac sodium group decreased by 2.5 times more than of the ARIL-1 group, and its value was close to the initial background. The group of rats treated with ARIL-1 had also the temperature reduction, but the average temperature at the end of the study was significantly different from the initial temperature (1.7°C).

Raleukin (29.2%) was 2.7 times inferior to diclofenac sodium (78.3%) by its average antipyretic activity in the therapeutic mode of introduction.

The studies have shown that the antipyretic effect of raleukin depends on the drug introduction mode. In the preventive mode of introduction raleukin greatly exceeded the reference drug, its activity was 63.6%, while the activity of diclofenac sodium was 37.5%. On the contrary, in the therapeutic mode raleukin was inferior to the reference drug. Its activity was 29.2%, while sodium diclofenac showed a significant antipyretic effect (78.3%), which was twice better than the action of raleukin in this mode.

CONCLUSIONS

Thus, it has been determined on the model of acetic acid writhings in mice that raleukin exhibits a moderate analgesic activity, which is considerably inferior to the one of the reference drug. However, it should be noted that raleukin has shown the analgesic effect in the dose of 3 mg/kg, which is 17 times less than the dose of analgin (50 mg/kg).

On the model of milk fever in rats raleukin has shown the expressed antipyretic effect in the preventive mode of introduction and the moderate one in the therapeutic mode. The difference in the intensity of the antipyretic activity of the drug in the various modes of introduction can probably be explained by the fact that during the therapeutic mode we deal with the consequences of the IL-1 propyrogenic effect as the result of the cytokine cascade launch; therefore, IL-1 receptor blocking in this case does not affect the intensity of the process, which has already started. And the preventive introduction of raleukin allows to delay the launch of the cytokine cascade [5, 6].

The pharmacological properties of raleukin determined allow to consider this drug to be a promising anti-inflammatory agent. It is reasonable to conduct further a profound experimental study with the aim to determine the peculiarities of its therapeutic effect.

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ПОРІВНЯЛЬНЕ ВИВЧЕННЯ АНАЛГЕТИЧНОЇ ТА ЖАРОЗНИЖУВАЛЬНОЇ ДІЇ РЕКОМБІНАНТНОГО АНТАГОНІСТА РЕЦЕПТОРІВ ІНТЕРЛЕЙКІНУ-1

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Ключові слова: ралейкін; оцтовокислі корчі; молочна лихоманка; жарознижувальна та аналгетична дія

В останні роки особлива увага привернута до проблеми створення та впровадження в медичну практику ефективних та безпечних протизапальних засобів. Відповідно до сучасних уявлень одним з пускових механізмів розвитку запального процесу є активація системи цитокінів. Отже, одним з перспективних напрямків сучасної протизапальної терапії є корекція кількісних, якісних і функціональних порушень цитокінової регуляції, зокрема шляхом блокади рецепторів, чутливих до цитокінів, зокрема, до інтерлейкіну-1 (ІЛ-1). Наведені результати порівняльного експериментального дослідження аналгезуючих та жарознижувальних властивостей оригінального рекомбінантного антагоніста рецепторів ІЛ-1 ралейкіну. Встановлено, що на моделі оцтовокислих корчів у мишей ралейкін проявив помірну аналгетичну активність, яка значно поступається дії анальгіну. Проте необхідно наголосити, що знеболювальну дію ралейкін проявив у дозі 3 мг/кг, яка в 17 разів менша, ніж доза референс-препарату. На моделі молочної лихоманки у щурів ралейкін чинив виражену жарознижувальну дію при профілактичному режимі введення та помірну – при лікувальному. Розбіжність у вираженості жарознижувальної активності препарату при різних режимах введення, ймовірно, можна пояснити тим, що при лікувальному режимі ми маємо справу з наслідками пропірогенного впливу ІЛ-1 внаслідок запуску цитокінового каскаду, тож блокування рецепторів ІЛ-1 у даному випадку ніяк не впливає на виразність процесу, який вже запущено. А профілактичне введення ралейкіну дозволяє загальмувати початок запуску цитокінового каскаду. Встановлені фармакологічні властивості ралейкіну дозволяють вважати даний препарат перспективним протизапальним засобом, що робить доцільним подальше поглиблене експериментальне вивчення з метою з`ясування особливостей його лікувальної дії.

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ АНАЛЬГЕЗИРУЮЩЕГО И ЖАРОПОНИЖАЮЩЕГО ДЕЙСТВИЯ РЕКОМБИНАНТНОГО АНТАГОНИСТА РЕЦЕПТОРОВ ИНТЕРЛЕЙКИНА-1

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В последние годы особое внимание приковано к проблеме создания и внедрения в медицинскую практику эффективных и безопасных противовоспалительных средств. Согласно современным представлениям одним из пусковых механизмов развития воспалительного процесса является активация системы цитокинов. Итак, одним из перспективных направлений современной противовоспалительной терапии является коррекция количественных, качественных и функциональных нарушений цитокиновой регуляции, а именно путем блокады рецепторов, чувствительных к цитокинам, в частности, к интерлейкину-1 (ИЛ-1). Приведены результаты сравнительного экспериментального исследования анальгезирующих и жаропонижающих свойств оригинального рекомбинантного антагониста рецепторов ИЛ-1 ралейкина. Установлено, что на модели уксуснокислых корчей у мышей ралейкин проявил умеренное анальгезирующее действие, которое значительно уступает анальгину. Однако необходимо отметить, что обезболивающее действие ралейкин проявил в дозе 3 мг/кг, которая в 17 раз меньше, чем доза референс-препарата. На модели молочной лихорадки у крыс ралейкин оказывал выраженное жаропонижающее действие при профилактическом режиме введения и умеренное – при лечебном. Разницу в выраженности жаропонижающей активности препарата при различных режимах введения, вероятно, можно объяснить тем, что при лечебном режиме мы имеем дело с последствиями пропирогенного влияния ИЛ-1 в результате запуска цитокинового каскада, поэтому блокирование рецепторов ИЛ-1 в данном случае никак не влияет на выраженность процесса, который уже запущен. А профилактическое введение ралейкина позволяет затормозить начало запуска цитокинового каскада. Установленные фармакологические свойства ралейкина позволяют считать данный препарат перспективным противовоспалительным средством, делают целесообразным дальнейшее углубленное экспериментальное изучение с целью выяснения особенностей его лечебного действия.

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