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Analysis of anesthesia effects of pharmacological drugs combinations for regional anesthesia in dentistry

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Abstract

Background: The effects of pharmacological preparations combinations, catecholamine levels (adrenaline, noradrenaline, dopamine), latent periods and nociceptive sensitivity threshold were studied in a model of regional anesthesia in experimental animals.

Material and methods: The analgesic effect of lidocaine anesthetic and its combinations with adrenoreceptor, histamine and dopamine receptors ligands were evaluated in «hot plate» test experiments in male Wistar rats before and in 15, 30, 60, 90 min after a local injection. Clinical and laboratory studies of catecholamine indexes in urine of laboratory animals have been carried out during infiltration anesthesia modeling by new medical combinations of pharmacological preparations.

Results: The data obtained in laboratory animals in the model of regional anesthesia at the combined use of lidocaine and dimedrol (diphenhydramine) indicate the effect of sensory perception suppression and demonstrate proposed pharmacological agents combinations efficacy. The single administration of lidocaine and its combinations with dimedrol, gromecin and diclofenac in quadriceps muscle of thigh (M. quadriceps femoris) in experimental animals results in hypoalgesic effect that was indicated by a significant ($p < 0,05$) increase of latent period of nociceptive response (LPNR) and pain reaction (LPPR) .

The adrenaline level values in the urine of a laboratory animal were the most informative during infiltration regional anesthesia modeling by pharmacological preparations combinations.

Conclusion: The ratio of lidocaine 2% hydrochloride and 1% dimedrol makes it possible to provide adequate, sufficient in depth and duration, controlled anesthesia, depending on the manipulation performed, which is a key point in dentist practice.

Introduction

Effective analgesia in the course of diagnostic and therapeutic manipulations is a key factor for a patient therapy. Pain sensations during dental treatment results in problems for both the patient and the physician, not allowing to conduct the full extent of interventions, reducing the quality of the performed manipulation, contributing to the formation of a negative dominant in the patient, up to «iatrogenic stress» caused by the influence of medical factors.

Adequate action is a pain relief, which allows intervening in the conditions of patient's emotional calmness, painless and without complications, i.e. comfortable for the patient and the physician. At present, almost all these conditions are attributable to the so-called combined anesthesia [1-11]. The main way is to study the combinations of already known pharmacopeia approved preparations with known pharmacological effect. The main requirements for local anesthetics are their efficacy and safety. Our attention was drawn to pharmacological preparations that are similar in their chemical structure and when mixed form a true, transparent and stable in time solution. The main goal of combined use of pharmacological agents (combinations) is to increase and expand the spectrum of therapeutic action and to reduce side effects.

In our case the interaction has the effect of synergy (amplification), e.g. simple summation (additive action) or potentiation, when the total effect exceeds the simple addition of analgesic (therapeutic) effects of each component. Presumably, this compatibility or combination of drugs could not only improve the efficacy of the local anesthetic, lidocaine, but also give it qualitatively new shades. The urgency of such work is determined by practical health care needs [1-11].

Study objective

To evaluate the effect of new pharmacological preparations combinations on the dynamics of biological fluids laboratory parameters and nociceptive responses of experimental animals during infiltration anesthesia modeling.

Methods

The experiments were carried out in accordance with international ethical and scientific quality standards for planning and conducting of animal studies [3, 12-15].

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The following pharmacological preparations were selected for the study: lidocaine hydrochloride (2% injectable solution, 2 ml N10), a universal local anesthetic of the amide series that induces all kinds of local anesthesia, an antiarrhythmic, class 1B; glycine (sublingual tablets, 100 mg), CNS neurotransmitter; dimedrol (1% injectable solution, 1 ml N10), a blocker of histamine H-1 receptors with anti-allergic activity; diclofenac (injectable solution 75 mg, 3 ml N10), a non-steroidal anti-inflammatory agent.

The combinations of the test substances solutions in a volume of 0.3 ml were introduced into the quadriceps femoris muscle (*M. quadriceps femoris*). Glycine was administered intragastrically by means of thin metal atraumatic tube.

The experiments were performed on 70 white male rats. The number and numerical composition of animal groups is the minimum that is necessary for experiments realization and is determined by the requirements and recommendations for preclinical studies of new pharmaceutical agents [3, 12-15]. The removal of animals from the experiment was carried out by intraperitoneal administration of sodium thiopental solution overdose.

Urine sampling for biochemical studies was performed in 2 and 24 hours after the administration of pharmaceuticals combinations.

The level of adrenaline, noradrenaline and dopamine was determined in urine by the method of enzyme linked immunosorbent assay. Determination of the concentration of hormones was carried out by test kits of «LDN» company (Germany), the results were expressed in ng/ml. The level of adrenaline and noradrenaline in the test samples was calculated from a calibration curve plotted against known standard concentrations with the help of SIRIO spectrophotometer (Italy).

Assessment of behavioral defense reactions and pain sensitivity in experimental animals under the conditions of application of anesthetics combinations affecting on adreno-, histamine, and dopamine receptors ligands was carried out by two tests: "Randall-Selitto" and "Hot plate" before injection of the studied drug combinations (control), and also in 5, 15, 30 and 45 minutes after injection [4,5]. Depending on the combination of pharmaceuticals and the type of research, 10 groups of 7 animals were created in each observation group.

The pain threshold was evaluated on the basis of registration of the latent period of rat nociceptive response (LPNR) on a thermal nonmechanical stimulus, i. e. on the minimum temperature at which the first defense reactions appear. LPNR was measured with an analgesimeter («Hot plate», Stoelting, USA).

The animal was placed on a metal plate heated to 50 ° C and the "start" button on the instrument panel was pressed. The latent time of withdrawal or licking of the hind limbs was noted. If the animal did not show activity for a 60-second interval, it was removed from the hot surface, and 60 seconds were taken as a latent time. LPNR was measured in seconds. After the control LPNR measurement, the animals were injected with the test combinations of substances with a repeated fourfold LPNR measurement.

In addition to the thermal stimulus, "Randall-Selitto" rat leg mechanical compression test was used, where mechanical stimulus was applied with increasing pressure on a rat leg, at which sensation of pain arose when the pressure reached a value sufficient to A-delta mechanoreceptors and C-polymodal nociceptors activation. The level of pain sensitivity in this test was determined in terms of the critical pressure magnitude on the plantar part of the animal's hind leg. To study the latent period of the pain reaction (LPPR, g) before and after pharmaceutical agents administration in animals by means of an electronic algesimeter (PanLab Harvard Apparatus, Spain), the pressure of the plastic cone (g/mm^2) on the foot was measured, which indicated a specific pain response (withdrawal of the leg or vocalization). The test was performed alternately on both hind legs of each animal. Animals that did not squeak up to the maximum allowable pressure were discarded [16-17].

Study design

Experimental animals (rats) were divided into groups, depending on the combinations of pharmacological agents (Table 1).

Statistical analysis

Statistical data processing was carried out with the help of «Statistica - 7», software packages of standard statistical and mathematical analysis, with the use of parametric and nonparametric criteria of mathematical statistics. In order to identify the pattern, a comprehensive intragroup and intergroup statistical analysis of the minimum, maximum and average values, central variants, average deviation and standard deviation was carried out. The confidence level was determined using the non-parametric Whitney-Mann test. Continuous covariates with a normal distribution were expressed as mean ± standard deviation (± Sx) and compared by Student's test within the group. Independent inter-group comparisons were calculated using one-way ANOVA test.

Results

The results of clinical and laboratory studies of catecholamine levels in urine of experimental animals are presented in the Table 2.

The highest adrenaline concentrations in rat urine were noted in the group of intact animals, the lowest and statistically significant differences were noted in the groups: "lidocaine + dimedrol", "lidocaine + diclofenac", "lidocaine + glycine", after 2 hours, compared to the control group. The highest levels of norepinephrine in animals urine were observed in the "intact" and "lidocaine" groups, the lowest in the groups: lidocaine + dimedrol "," lidocaine + diclofenac ","lidocaine + glycine". The highest values of dopamine in animal urine were also noted in the "intact" and "lidocaine" groups, the smallest in the "lidocaine + dimedrol", "lidocaine + diclofenac", "lidocaine + glycine" groups.

After single intramuscular and intragastric (glycine) administration of the investigated combinations of pharmacological preparations to the white rats at the above-mentioned doses no changes in the physiological parameters were observed during the observation period. Appearance, behavior, condition of body hair coat, skin and visible mucous membranes of experimental animals were not changed during the whole period of observation.

In experiments on male Wistar rats in the "hot plate" and "Randall-Selitto" tests an analgesic effect of lidocaine anesthetic and its combinations with adrenoreceptor, histamine and dopamine receptors ligands were evaluated before and in 5, 15, 30, 45 min after local injections. The results are shown in Table 3.

Analyzing the obtained experimental data, we can conclude that the maximum depth of anesthesia in the observation groups is reached after 15, 30, 45 min ($p < 0.05$).

Experiments on white male rats showed that:

- the "lidocaine + dimedrol" combination induces an effect close to the effect of pure lidocaine, but the effect is more pronounced;

Table 1. The allocation of animals in the groups.

Item	Test and study design	Animal species and number	Observation time
1	**I group (lidocaine)**		
1.1.	Injection of 0,3 ml of lidocaine into the quadriceps femoris muscle (IM)	Rats, n=14	5, 15, 30, 45 min (LPNR, LPPR) (2 h, 24 h, ELUA)
2	**II group (lidocaine + dimedrol)**		
2.1.	Injection of 0,15 ml of lidocaine + 0,15 ml of dimedrol (IM)	Rats, n=14	5, 15, 30, 45 min (LPNR, LPPR) (2 h, 24 h, ELUA)
3	**III group (lidocaine + diclofenac)**		
3.1.	Injection of 0,15 ml of lidocaine + 0,15 ml of diclofenac (IM)	Rats, n=14	5, 15, 30, 45 min (LPNR, LPPR) (2 h, 24 h, ELUA)
4	**IV group (lidocaine + glycine)**		
4.1	Injection of 5 mg of glycine (IG) + 0,15 ml of lidocaine (IM)	Rats, n=14	5, 15, 30, 45 min (LPNR, LPPR) (2 h, 24 h, ELUA)
V group (control)			
5	Injection of 0,3 ml vehicle (IM)	Rats, n=14	5, 15, 30, 45 min (LPNR, LPPR (2 h, 24 h, ELUA)

Note: ELUA – enzyme-linked urine assay

Table 2. Clinical and laboratory catecholamine's values in experimental animal's urine ($\overline{X} \pm Sx$)

№ п/п	Animal group	Adrenaline ng/ml, after 2 h	Adrenaline ng/ml, after 24 h	Noradrenaline, ng/ml, after 2 h	Noradrenaline, ng/ml, after 24 h	Dopamine ng/ml, after 2 h	Dopamine ng/ml, after 24 h
1	Intact	123,84 ± 21,7	18,48 ± 4,5	525,25 ± 151,8	80,09 ± 18,78	1563,09 ± 248,7	236,74 ± 68,12
2	Lidocaine	58,08 ± 17,5*	14,4 ± 4,2	577,105 ± 167,312	167,7 ± 51,9	1119,56 ± 342,5	307,56 ± 99,7
3	Lidocaine+ dimedrol	32,38 ± 9,7*	4,29 ± 1,3*	246,96 ± 77,3	32,17 ± 9,8	837,03 ± 233,5	109,61 ± 27,7
4	Lidocaine+ diclofenac	32,12 ± 5,3*	3,145 ± 0,7*	267,56 ± 69,3	25,5 ± 5,8*	1065,59 ± 315.3	104,9 ± 28,7
5	Lidocaine+ glycine	34,2 ± 6,5*	4,34 ± 1,2*	221,43 ± 55,8	25,27 ± 7,1*	834,07 ± 262,3	95,07 ± 22,7

Note: *$p<0,05$

Table 3. Effects of pharmacological preparations combinations on rat nociceptive response.

Medicinal products, time	Number of rats, (n)	Latent period of nociceptive response (LPNR) (s)	Latent period of pain reaction (LPPR) (g)
vehicle			
before administration	7	7,00 ± 2.41	127,9 ± 23,5
5 min	7	4,36 ± 1,96	119,3 ± 13,9
15 min	7	7,01 ± 1,98	134,6 ± 40
30 min	7	5,70 ± 2,23	141,1 ± 27,6
45 min	7	5,16 ± 1,60	134,1 ± 24,2
lidocaine			
before administration	7	6,85 ± 3,10	114,8 ± 37,6
5 min	7	7,77 ± 2,06**	162 ± 80,4
15 min	7	10,53 ± 2,94*	163,4 ± 105
30 min	7	10,30 ± 1,97**	211 ± 84,3*
45 min	7	10,68 ± 2,85**	198,8 ± 79,1*
lidocaine + dimedrol			
before administration	7	8,16 ± 1,95	117,7 ± 32,5
5 min	7	8,18 ± 1,39**	169,6 ±38,9*
15 min	7	6,89 ± 2,00	196,3 ± 34*
30 min	7	6,70 ± 1,57	235 ± 53,3*
45 min	7	5,48 ± 1,90	258 ± 41,1*
lidocaine + diclofenac			
before administration	7	7,23±2,70	111,9 ± 42,2
5 min	7	8,08 ± 1,86**	204,8 ± 80*
15 min	7	7,78 ± 2,64	157,8 ± 84,1
30 min	7	6,87 ± 2,03	189,9 ± 98,8
45 min	7	6,38 ± 1,14	172,8 ± 92,7
lidocaine + glycine			
before administration	7	5,00 ±1,83	132,3 ± 15
5 min	7	10,30 ± 5,30*	163,6 ± 57
15 min	7	8,32 ± 3,50	238,1 ± 36,8*
30 min	7	6,62 ± 2,60	239,3 ± 61,9*
45 min	7	6,70 ± 2,30	234 ± 50*
* – statistically significant at $p < 0,05$ relative to the control group; ** – statistically significant at $p < 0,01$ relative to the control group;			

- the combination of "lidocaine + diphenhydramine" results in effect close to the effect of lidocaine, but it is more pronounced;
- the combination "lidocaine + glycine" is characterized by a short-term analgesic effect;
- the combination "lidocaine + diclofenac" causes the effect of sensory sensitivity suppression, similar to the effect of pure lidocaine, but the effect is more prolonged in comparison with other mixtures.

An analysis of the experimental data made it possible to conclude that after a single intramuscular injection of lidocaine and combinations of lidocaine preparations with dimedrol, glycine and diclofenac, a hypoalgesic effect was noted in male rats, as indicated by a significant ($p < 0.05$) increase in the latent period of the nociceptive response.

Conclusions

The data obtained in laboratory animals in the model of regional anesthesia with the combined use of lidocaine and dimedrol indicate the effect of sensory perception suppression and demonstrate the efficacy of the proposed pharmacological agents' combinations. The ratio 2% lidocaine hydrochloride / 1% dimedrol provides adequate, sufficient in depth and duration, controlled anesthesia depending on the manipulation performed, which is a key point in the practice of a dentist.

The most informative and statistically reliable indicator is an adrenaline level in the laboratory animal urine at infiltration regional anesthesia modeling with pharmacological preparations combinations.

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This study was approved by the ethics committee of the Institute.

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