



Efficacy of liposomal dosage forms and hyperosmolar salines in experimental pharmacotherapy of acute lung injury

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Abstract

Introduction: Hypertonic sodium chloride solutions and liposomal drugs with pulmotropic effect are of great interest for the treatment of acute lung injury (ALI). The results of the studies on the efficacy of hypertonic solutions and liposomes in ALI treatment are currently controversial.

Materials and methods: For the experiment, liposomes with dexamethasone, N-acetylcysteine (NAC), aprotinin and dye Cyanine-7 (Cy-7) were obtained. A liposome analysis was performed by means of spectrophotometry. ALI was modeled in rats by the administration of the damaging agents into the trachea. The experimental agents were injected once intravenously after the modeling of ALI. For experimental therapy used liposomal agents, 7.5% hypertonic saline (HS) and HyperHAES solutions in the respective groups. The efficacy of the therapy was assessed by the survival of animals, functional indicators of the cardiovascular and respiratory systems, and by the lung-body ratio. The biodistribution of liposomes after intravenous administration was investigated in mice through using a fluorescent dye Cy-7. The biodistribution of liposomes with Cy-7 was assessed using bioimaging according to the fluorescence intensity of internal organs (lungs, liver, and kidneys) and blood, expressed as dye concentration according to the calibration dependence of dye concentration on fluorescence intensity.

Results and discussion: All the studied liposomal drugs were effective for the pharmacological correction of ALI. Hypertonic solutions, unlike liposomal drugs, were less likely to prevent the development of pulmonary edema. All the studied therapeutic agents increased the survival rate of the laboratory animals with ALI. The most effective experimental agent was liposomal dexamethasone. The use of drugs in form of simple liposomes with average diameter of 350 nm provided for a higher concentration of the drug in the lungs within the first 40 minutes after intravenous administration.

Conclusion: Intravenous administration of liposomal forms is promising for the pharmacotherapy of acute lung injury.

Keywords

N-acetylcysteine, acute lung injury, aprotinin, Cyanine-7, dexamethasone, HyperHAES, hypertonic saline, liposomes.

Introduction

Among the direct causes of acute lung injury (ALI), the most common one is the aspiration of various exogenous and endogenous substances (stomach contents) into the lower airways (Avdeev 2008).

Interest towards **hypertonic saline (HS) of sodium chloride**, as an anti-edematous and anti-inflammatory agent in acute lung injury has appeared only recently (Fominisky 2013). The therapeutic effect of **HS** in ALI was shown in cell culture and in vivo experiments, and it was associated with the suppression of neutrophil activation (Holms et al. 2015). The mechanism of **HS** action in case of ALI is associated with inhibition of neutrophil responses (chemotaxis, phagocytosis, etc.) (Inoue et al. 2011). Unlike colloids, **hypertonic sodium chloride solution** has an advantage that the limited volume of liquid is injected, and the alveolar-capillary barrier is increased. But the data about this remains controversial (Bulger et al. 2011, Roch et al. 2013). In addition, the effectiveness of the combination of **HS** sodium chloride with **hydroxyethyl starch (HES)** solutions as a means to prevent impairments of the oxygenating function of the lungs and to improve oxygen delivery to tissues is confirmed (Yu et al. 2012a). The combination of **HS** and **HES** inhibits neutrophil adhesion to the endothelium and reduces the production of interleukins when administered intravenously (Safdar et al. 2005, Yu et al. 2012a). Recent studies have shown an increasing interest towards hyperosmolar solutions in which plasma hyper osmolarity and hypernatremia are assessed as factors contributing to the therapeutic effect in ALI (Bihari et al. 2016).

Liposomes are well-studied drug carriers. They allow encapsulating both hydrophilic and lipophilic substances (Bhardwaj and Burgess 2010). They have a shell – an artificial membrane – and are used for the selective delivery of therapeutic drugs into various tissues for create there an effective concentration of drug (Baryshnikov 2012). The relative ease of including therapeutic agents in liposomes, the possibility of direct delivery of liposomes to the appropriate part of the body, the relative non-immunogenicity, and low toxicity made liposomes very appealing for drug delivery to the lungs (Bailey and Berkland 2009). To have liposomal dosage forms target lungs, the so-called “vascular targeting” strategy can be used (Carnemolla et al. 2013). This experimental strategy allows delivery inside or through endothelial cells (Guo et al. 2014).

When studying the biodistribution of simple liposomes in damaged lungs, they were found to accumulate differently in healthy and affected areas due to the different intensity of blood flow and damage to the capillaries (Brenner et al. 2018). To confirm the significant influence of size on liposome accumulation in lungs, there are relevant studies on ALI models and intact lungs. All of them show that the longer liposomes circulate in the bloodstream, the more they accumulate in lungs (Wei et al. 2017).

A promising form of treatment for ALI is **N-acetylcysteine (NAC)**. In isolated lungs and in vivo, it was shown

that **NAC** reduces cell apoptosis of lung tissue (Chiang et al. 2012), while liposomal **NAC** had better pharmacokinetics and efficacy than free **NAC**, especially under systemic oxidative stress (Xu et al. 2012). Recently, there have been some studies on liposomal **NAC** in ALI, but only as an inhalant (Ourique et al. 2014), or as a means to prevent the development of ALI (Mitsopoulos et al. 2008). There have been no experimental studies considering the therapeutic efficacy of liposomal **NAC** in aspiration lung injury.

Recently, a number of studies have noted the efficacy of protease inhibitors such as **aprotinin**, **ulinastatin** when administered intravenously in relation to improving the morphological picture of lung tissue, oxygenation level, reducing pulmonary edema in ALI, but without describing their mechanism of action. Currently, there are some assumptions about possibility of using protease inhibitors in ALI treatment. They concern the reduction of leukocyte infiltration of lung tissue, the level of interleukins and, accordingly, the pulmonary edema (Cao et al. 2018). The mechanism of therapeutic effect of **aprotinin** in ALI is associated with inhibition of proteolytic enzymes secreted by neutrophils (Li et al. 2015). Besides **aprotinin**, in rats with experimental acute pancreatitis, in addition to reducing the level of inflammatory mediators, also reduced the level of peroxynitrite and reactive oxygen species and, like **ulinastatin**, reduced leukocyte infiltration of lung tissue and the degree of pulmonary edema (Cao et al. 2018). Such data make it promising to study the use of the liposomal form of protease inhibitors for ALI treatment.

Due to the highly efficient loading of **dexamethasone** in liposomes, reaching 80–90% (Chen et al. 2013), liposomes with this drug are a very promising cure for ALI. Studies of the pharmacokinetics of liposomes with **dexamethasone** when administered intravenously showed a 5-fold higher accumulation of **dexamethasone** in lungs compared with a conventional solution of the same concentration (Chen et al. 2013). Recent studies confirm a more pronounced anti-inflammatory effect of liposomes with **dexamethasone** on lungs, due to a longer contact with the endothelium of pulmonary capillaries and the accumulation of a higher concentration the active substance in lungs (Li et al. 2018).

Biodistribution of liposomes in living objects can be investigated using optical imaging in the near infrared range, which eliminates absorption and scattering in biological tissues (Guan et al. 2017, Ravoori et al. 2016). The fluorescent dye **Cyanine-7 (Cy-7)** is suitable for such studies, because **Cy-7** gets well anchored in liposome membranes, because it has hydrophobic properties (Desu et al. 2016). Cyanine dye is loaded into liposomes with quite high efficiency, up to 90% (Zou et al. 2013). If it is necessary to investigate the biodistribution and accumulation of nanoparticles in internal organs, the use of **Cy-7** as a fluorescent marker for liposomes is more preferable. This is primarily due not only to the emission wavelength, but also to the stability of the fluorescent signal after its administration in vivo (Yu et al. 2012b). The near-infrared flu-

orescent imaging is currently the most convenient way to monitor the dynamics of liposomal dosage forms in vivo because it is a non-invasive and non-ionizing method that allows for deep visualization of tissues, including lungs (Mizuno et al. 2009), and for investigation of the pharmacokinetics of drugs that are incorporated into nanocarriers. With the help of preliminary calibration calculations, at present, the target properties of fluorescent liposomes can be quantified in relation to lungs tissue and the main pharmacokinetic indicators (Rajasekaran et al. 2015).

Materials and methods

Characteristics of hyperosmolar solutions

HyperHAES – solution for infusions produced by Fresenius Kabi Deutschland GmbH, Bad Homburg (Germany). The combination of 7.2% solution of NaCl and 6% HES with a molecular weight of 200000 Da and a degree of molar substitution of 0.5 (pentastarch) 60 g/L; pH 3.5–6.0; osmolarity – 2464 mOsmol/L; Na 1232 mmol/L; Cl 1232 mmol/L (Fominsky 2013).

7.5% NaCl solution, which was obtained in the chemical laboratory of Ogarev Mordovia State University from purified water and NaCl (c.p.), followed by autoclave sterilization at a temperature of 120 °C and under pressure of 1 atm. Ingredients: Na 1283 mmol/L, Cl 1283 mmol/L; pH 4–6.0; osmolarity – 2566 mOsmol/L.

Production and analysis of liposomes

Liposomes were obtained by phase inversion from lecithin (**phosphatidylcholine**) EPCS 10 8018-1/130, Lipoid (Germany) and **cholesterol** Avanti Polar Lipids, Inc. (USA). To create liposomes, a Heidolph rotary evaporator was used (Germany). A dispersion of multilamellar vesicles was passed through a polycarbonate filter with a pore diameter of 400 nm using an LIPEX™ extruder (Canada).

Purification of liposomal suspension from substances not included in liposomes was carried out using dialysis in an inert gas atmosphere under pressure of 0.3 MPa, as well with use of gel filtration, for which **Sephadex G-75** gel was used. Liposomes sizes were estimated using dynamic light scattering on a NANO-flex analyzer (Microtrac Inc., USA). The amount of drug substance in liposomes was estimated by spectrophotometry using a Shimadzu spectrophotometer (Japan).

Cyanine-7 dye made by Biotech-Industry (Russia) was included in the lipid film before the formation of liposomal vesicles and it was incorporated into liposome membranes. The efficiency of dye incorporation into liposomes was 98%, the concentration of **Cy-7** in a liposomal suspension was 23 µg/ml, and the average diameter of liposomal vesicles was 313±2 nm.

When producing liposomal **NAC** (L-NAC), the lipid film was hydrated with 100 mg/ml of Flumucil (N-acetylcysteine solution), (Zambon, Italy). Free **NAC**

was separated from L-NAC through gel filtration. The amount of **NAC** incorporated into liposomes was determined spectrophotometrically by means of the reaction with **FeCl₃** and **1,10-phenanthroline** at a wavelength of 510 nm, using a previously constructed calibration curve. The degree of **NAC** inclusion into liposomes was 12.5±2.5%. The average size of the obtained liposomes was 430±40 nm.

For obtaining liposomes with **aprotinin**, “**Gordox**” manufactured by Gedeon Richter Corp. (Hungary) was used, an injection solution, containing 10.000 KIU/ml of **aprotinin**. For the quantitative analysis of the obtained liposomes, a solution of **Cy-7** with a concentration of 27 µg/ml was used. **Cy-7** served as an optical marker of **aprotinin** molecules in the quantitative analysis of liposomes. Liposomes were purified from free dye and the dye which had failed to bind to **aprotinin** by means of dialysis method. According to the results of spectrophotometry ($\lambda = 760$ nm), 252.156 µg of **Cy-7** dye binds to 120.000 KIU of **aprotinin**, the binding efficiency was 77.8%. Purification of liposomes from the free complex aprotinin-Cy-7 was carried out by gel filtration. The results of the quantitative analysis of liposomes with **aprotinin** showed a 3184.8 KIU/ml concentration of the drug in liposomal suspension, with entrapment efficiency of 31.85%. Liposomes containing **aprotinin** were of 312.3±12.5 nm in size.

For producing liposomes, “**Dexametstone**” was used solution for injection 4mg/ml, KRKA Novo mesto, Slovenia. Lipid film dried on a rotary evaporator was hydrated with a **hypertonic solution of sodium chloride** (75 mg/ml), containing **dexamethasone** at a concentration of 4 mg/ml. Purification of liposomes from free **dexamethasone** was performed through dialysis. Quantitative determination of **dexamethasone** content in liposomes was made by means of spectrophotometry at $\lambda=241$ nm. The average diameter of liposomes was 320±50 nm. The concentration of **dexamethasone** in the purified liposome dispersion was 2.9795 mg/ml±0.015 mg/ml. The drug entrapment efficiency in liposomes was 74.5%±0.4%.

Modeling acute lung injury and anesthesia

In the experiment, two models of ALI were used. The first model involved injection of **acetone** in an amount of 0.1 mg/kg into the trachea (Kulikov et al. 2015), the second model involved intratracheal administration of **acidin-pepsin** solution (1 tablet per 0.5 ml of **normal saline**) in the amount 0.03 ml per one animal. For the experiment, white non-linear rats of both sexes weighing 220–300 g were used from Stolbovaya nursery (Research Center for Biomedical Technologies of the Russian Academy of Medical Sciences). For the experiment, “**Acidin-Pepsin**” tablets containing 200 mg of betaine hydrochloride and 50 mg of pepsin were used, (Belmedpreparaty, Republic of Belarus) (Moroz et al. 2010). The procedure for ALI modeling was an operation during which an incision of about 5 mm in size was made in the rat’s neck area to get access to the trachea, above the thyroid gland, and then a

18G catheter was inserted into the trachea. The catheter was filled with the appropriate agents, then they were administered into the trachea, after which the catheter was removed and the incision was sutured. To prevent reflex respiratory arrest, artificial lung ventilation was used, with the parameters of 500 ml/min, respiratory rate (RR) of 60 per minute, and respiratory volume of 5 ml/kg of animal weight. The surgical manipulations with animals, including euthanasia, were performed under injection anesthesia (8 mg/kg of **Rometar** and 20 mg/kg of **Zoletil** intravenously into the lateral vein of the tail). The experiment was carried out after the approval by the local ethics committee in accordance with the rules for working with laboratory animals formulated in Directive 2010/63/ EU of the European Parliament and the Council of the European Union on the Protection of Animals Used for Scientific Purposes (Directive 2010).

Experimental protocol

After ALI modeling, all the animals were injected with **ceftriaxone** (bottled powder, 1g. for preparing a solution for intramuscular and intravenous administration, Biosintez, Russia) at a dose of 200 mg/kg, intramuscularly once a day for 6 days (for basic support in ALI) (Galvão et. al. 2016). For the two models of ALI, 5 groups of animals were formed. The animals of Group 1 (control 1) received no other treatment. The animals of the experimental groups received the therapy, which consisted of a single intravenous administration of the test agents 20 minutes after intratracheal administration of the damaging agent. The rats in Group 2 (control 2) were injected with **dexamethasone** at a dose of 6 mg/kg. In Group 3, the animals were intravenously injected with 75mg/ml (7.5%) **hypertonic solution of sodium chloride**. In Group 4, the animals were intravenously administered **HyperHAES** solution as a single dose. Hypertonic solutions were administered at a dose of 4 ml/kg (Fominsky 2013). In Group 5, during **acetone** aspiration, the animals were injected intravenously with 0.5 ml of liposomal suspension with **N-acetylcysteine** (NAC average dose of 25–30 mg/kg), and in **acidin-pepsin** aspiration, liposomal **aprotinin** (L-aprotinin) was injected intravenously at a dose of 12.000 KIU/kg. A separate group consisted of intact rats.

Therapeutic efficacy of liposomal form of **dexamethasone** in hyperosmolar aqueous medium HLD was investigated on ALI model, using **acidin-pepsin**. The animals were once intravenously injected with 0.5–0.6 ml hyperosmolar liposomes at a **dexamethasone** dose of 6 mg/kg, 5 minutes after the pathology modeling. Treatment efficacy was compared with that in case of intravenous administration of a **dexamethasone** solution at a dose of 6 mg/kg in combination with **hypertonic (7.5%) sodium chloride solution** (HS+Dex). The dose of **hypertonic saline** with intravenous administration of liposomes with and in the comparison therapy was 2 ml/kg.

Evaluating the efficacy of the ALI experimental therapy

Functional indicators

Registration of physiological parameters was carried out by means of Biopac MP 150 (BIOPAC Systems, Inc., USA). The animals were evaluated in terms of their external respiration parameters: hemoglobin saturation (SpO₂), respiratory rate (RR); indicators of the cardiovascular system: the heart rate (HR); blood pressure (BP): systolic and diastolic. The pressure was measured noninvasively on the tail. The functional indicators in all experimental groups were recorded at 60 min, 24 hours and 6 hours after the modeling of ALI. In the study of the therapeutic efficacy of liposomal **dexamethasone**, the physiological parameters were recorded 20 minutes before the ALI modeling, 5 minutes afterwards and then further 5, 30, 60, 120, 240 minutes, 24 hours and 6 days after the time of applying the treatment.

Calculation of survival

To assess the effectiveness of the compared therapy methods, mortality was recorded within 6 days from the moment of ALI modeling, the percentage of surviving animals (survival rate) was determined by the formula $(X_s/X_c)/100\%$, where X_s is the number of animals surviving on the 6th day of the experiment, X_c – the number of animals in the group.

Analysis of the degree of pulmonary edema

For the experiment, similar experimental groups were formed, each containing 10 animals. Part of the animals were sacrificed on the first day, whereas the others – on the 6th day after the start of the experiment under anesthesia by careful simultaneous sectioning carotid arteries without traumatizing the trachea (Gushchin and Muzhikyan 2014). In the animals, the lungs were removed and weighed, followed by determining the degree of pulmonary edema – Lung-body (LB) ratio. LB ratio was calculated by the formula: weight of lungs/weight of animal×1000 = units (Torkunov 2007).

In vivo imaging biodistribution of liposomes

For calibrating the method, in advance the curve showing the dependence of concentration of liposomal and free dye **Cy-7** on fluorescence intensity was constructed. For this purpose, various dilutions of **Cy-7** dye had been prepared.

The study was carried out on white outbred male mice weighing 20–23 g. Liposomes with fluorescent dye **Cy-7**

were intravenously administered in the lateral tail vein in a volume of 0.2 ml per animal. The animals were sacrificed by decapitation under general anesthesia 2.5, 5, 10, 15, 20, 30, 40 and 60 min after intravenous administration. The internal organs (liver, lungs and kidneys) were removed and weighed; also 0.2 ml of blood was taken. The organs and blood were placed in IVIS Lumina II apparatus (Caliper, USA), and fluorescence intensity was recorded. Fluorescence intensity was converted to the number of micrograms of the dye per 1 gram of organ tissue. The conversion was carried out according to the calibration curve and taking into account the density of the target organ tissue. Density of the organs was calculated experimentally in 10 individual mice of the same weight – 22 g. The number of the animals for each time point in both series of was experiments was $n=5$.

Statistical analysis

The observed differences in numerical values were evaluated using Student's t-test and χ -square test. A conclusion about statistically significant differences was made with a significance level (p) <0.05.

Results

Twenty-four hours after acetone aspiration, 61.53% of the animals which had been administered only with an antibiotic died. Mortality in the group of animals treated with dexamethasone was 30% (Fig. 1). Mortality in the groups with a single administration of hypertonic saline and HyperHAES solution was 41.6% and 25%, respectively, which, in case of administering HyperHAES, was significantly less than that in the group without experimental treatment. Mortality in rats that had received a single dose of liposomal NAC was 14.3%, which was significantly lower than that in the control, where only one antibiotic had been used. On the 2nd day of observation, the number of deaths in all experimental groups increased. In the control group, mortality was 76.9%, in the group of dexa-

methasone – 40%, and after administration of hypertonic saline solution and HyperHAES – 47.1% and 37.5%, respectively (Fig. 1). On the 2nd day after application of liposomal NAC, 21.4% of rats died. The values reflecting mortality rates in the groups where the treatment had been carried out, except for the group with hypertonic sodium chloride, were significantly lower than that in the group where experimental therapy had not been carried out. On the 3rd day, all the animals in the control group died (Fig. 1). The 3rd day also saw a mortality rate in the group after application of liposomal NAC increasing to 28.6%. The mortality rate was less than that in the control group without experimental treatment.

Saturation of blood hemoglobin (SpO_2) in the animals 1 hour after acetone aspiration reduced from $95.76 \pm 0.63\%$ in the normal condition to $78.4 \pm 1.67\%$ in the control group (Fig. 2). Under the influence of experimental therapy and comparing therapy with dexamethasone, the saturation was higher in when compared to the group, in which only support antibiotic therapy had been used. In the dexamethasone group, the hemoglobin saturation was $86.8 \pm 2.2\%$ one hour later. The highest saturation was in a group with HS $87.7 \pm 1.3\%$, with a solution HyperHAES – $85.8 \pm 2.0\%$. L-NAC supported hemoglobin saturation level – $85.7 \pm 1.3\%$.

One day after aspiration of acetone in the control group, the level of SpO_2 was $86.0 \pm 5.3\%$ (Fig. 3). The support of a sufficiently high level of oxygenation was provided with hyperosmolar solutions and dexamethasone. After administration of dexamethasone, the level of SpO_2 was $94.0 \pm 1.34\%$. After administration of HS and HyperHAES, the levels of SpO_2 were $93.7 \pm 3.2\%$ and $90.7 \pm 2.3\%$, respectively: At the same time, L-NAC was inferior to dexamethasone in efficacy at the saturation level of $88.3 \pm 1.91\%$ (Fig. 3).

The figure shows that on the 6th day, there was no difference between the levels of SpO_2 in experimental therapy with hyperosmolar solutions and in the treatment of L-NAC, the levels remaining reduced relative to the norm (Fig. 4). Hemoglobin saturation levels in groups with dexamethasone and L-NAC were $85.8 \pm 1.3\%$ and $88.0 \pm 2.5\%$, respectively, and in groups with HS and

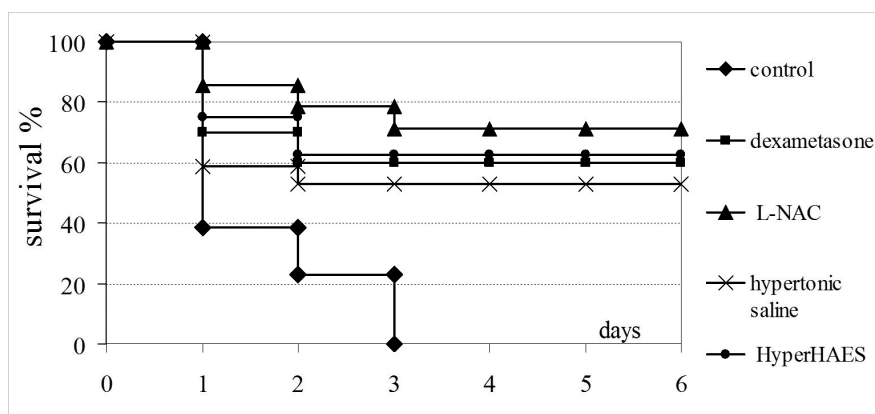


Figure 1. Animal survival in acute lung injury caused by aspiration of acetone after using various types of therapy.

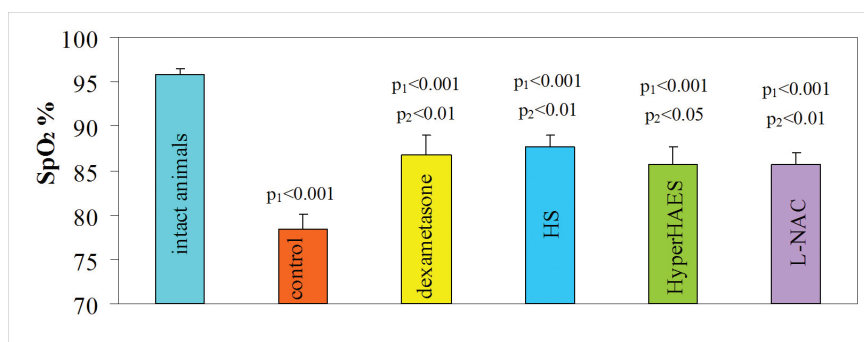


Figure 2. The changes of blood hemoglobin saturation in rats 1 hour after administration of the studied agents on the background of acute lung injury. Note: p_1 – significance of differences, calculated in relation to the group of intact animals; p_2 – in relation to control group 1 (0.1 ml/kg of acetone intratracheally).

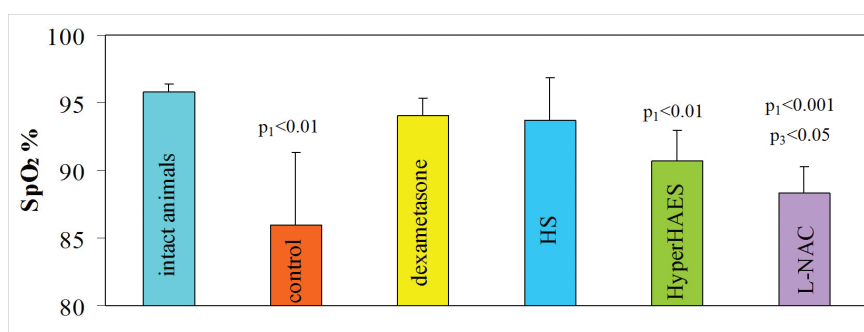


Figure 3. The changes in blood hemoglobin saturation levels in rats 24 hours after administration of the studied agents on the background of acute lung injury. Note: p_1 – significance of differences, calculated in relation to the group of intact animals. p_3 – in relation to the control group 2 (dexamethasone 6 mg/kg, intravenously, once + ceftriaxone 200 mg/kg).

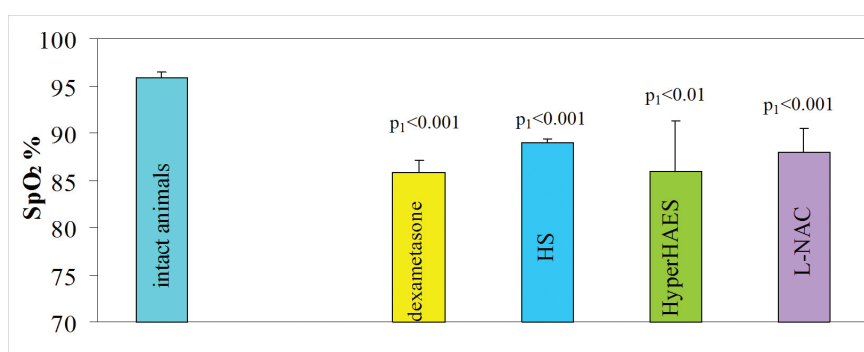


Figure 4. The changes in blood hemoglobin saturation levels in rats 6 days after administration of the studied agents on the background of acute lung injury. Note: p_1 – significance of differences, calculated in relation to the group of intact animals.

HyperHAES– $89.0 \pm 1.2\%$ and $86.0 \pm 5.3\%$. In the group with L-NAC, the SpO₂ level tended to increase, whereas in the others – to decrease (Fig. 4).

In the intact animals, RR was 83.1 ± 6.1 breaths per minute (1/m). This indicator shows that L-NAC together with HS and HyperHAES kept RR at the level corresponding to that of the intact animals – about 80–90/min (Table 1) 1 hour after their application. Twenty-four hours later, the respiration rate in the group where the dexamethasone was used remained 27.7%

higher than that in the intact animals and 44.9% higher than that in the group where HS had been administered (Table 1).

RR on the 6th day of the experiment in the group of animals, where HyperHAES solution had been used, was 28.9% lower than that in the group after the administration of dexamethasone. In the group after administration of liposomal NAC, RR in rats was significantly lower than that in the group of animals treated with dexamethasone – by 23.4% (Table 1).

Table 1. Changes in functional indicators in rats, with acute lung injury caused by aspiration of acetone after administration of the compared means of therapy ($M \pm m$, $n=10$).

Time	Indicators	Groups of experimental therapy				
		Control	Dexametason	HS	HyperHAES	L-NAC
1 hour	RR 1/min	56.5±7.6	106.1±6.8	80.6±7.4	89.0±6.3	98.0±7.9
		$p_1 < 0.05$	$p_{1,2} < 0.05$	$p_{2,3} < 0.05$	$p_2 < 0.05$	$p_2 < 0.05$
	HR 1/min	284.2±24.8	268.4±23.7	262.0±17.2	303.8±10.6	334.4±14.6
		$p_1 < 0.05$	$p_1 < 0.05$	$p_1 < 0.05$	$p_{1,4} < 0.05$	$p_{3,4} < 0.05$
Systolic BP mm Hg.		72.0±13.0	87.6±9.6	89.7±5.3	95.4±5.6	105.1±5.1
		$p_1 < 0.05$	$p_1 < 0.05$	$p_1 < 0.05$	$p_1 < 0.05$	$p_{1,2} < 0.05$
Diastolic BP mm Hg		39.1±8.4	55.4±7.2	58.5±4.4	61.8±4.1	73.9±5.2
		$p_1 < 0.05$	$p_1 < 0.05$	$p_1 < 0.05$	$p_{1,2} < 0.05$	$p_{1,2,4} < 0.05$
24 hours	RR 1/min	86.3±6.0	106.1±9.5	73.2±6.7	79.7±2.9	82.0±12.8
			$p_1 < 0.05$	$p_3 < 0.05$		
	HR 1/min	400.2±22.1	323±18.1	312.3±17.1	392±19.0	363.3±19.2
			$p_2 < 0.05$	$p_2 < 0.05$	$p_4 < 0.05$	
Systolic BP mm Hg		93.0±27.0	127.4±8.1	115.7±15.4	133.0±20.0	102.3±11.0
		$p_1 < 0.05$				$p_1 < 0.05$
Diastolic BP mm Hg		70.5±20.6	92.6±6.7	80.0±13.0	95.0±8.0	74.6±10.2
						$p_1 < 0.05$
6 days	RR 1/min	–	101.0±6.5	90.7±16.6	71.8±13.0	77.3±7.1
					$p_3 < 0.05$	$p_3 < 0.05$
	HR 1/min	–	396.8±20.5	402.8±16.0	347.4±25.7	357±18.2
Systolic BP mm Hg		–	133.0±10.9	107.6±10.1	101.6±16.9	144.3±20.8
				$p_1 < 0.05$	$p_1 < 0.05$	
Diastolic BP mm Hg		–	98.3±6.9	65.4±7.3	65.8±15.8	116.0±16.9
				$p_{1,3} < 0.05$	$p_1 < 0.05$	$p_4 < 0.05$

Note: p_1 – significance of differences, calculated in relation to the group of intact animals; p_2 – in relation to control group 1 (acetone intratracheally 0.1 ml/kg); p_3 – in relation to control group 2 (dexamethasone 6 mg/kg, intravenously, once + ceftriaxone 200 mg/kg); p_4 – in relation to the group treated with hypertonic saline intravenously once 4 ml/kg + ceftriaxone 200 mg/kg; HS – hypertonic saline; L-NAC – liposomal NAC; RR – respiratory rate; HR – heart rate; BP – blood pressure.

HR of healthy rats with appropriate anesthesia was 353.7 ± 19.4 beats per minute (1/min). The intravenous administration of L-NAC clearly affected the heart rate 1 hour after administration. The heart rate increased to 334.0 ± 15.0 /min. At the same time, the heart rate remained low in other types of therapy, which makes it impossible to talk about correcting this indicator with dexamethasone and hypertonic solutions in this model (Table 1).

In the intact rats, blood pressure was $136.7 \pm 6.0/97.7 \pm 4.9$ mm Hg. None of the therapeutic regimens showed any hypertensive effect 1 hour after the time the aspiration of acetone, which was not typical of drugs, such as dexamethasone and hypertonic solutions based on sodium chloride. At the same time, L-NAC caused no increase in blood pressure to reach the level of the intact animals (Table 1). However, systolic and diastolic BP levels 1 hour after administration of L-NAC were significantly higher than the levels of BP after acetone aspiration without the experimental therapy.

Maintaining a higher blood pressure after the administration of L-NAC compared to that when using HyperHAES solution is an interesting fact, possibly related to the effect of the liposomes, which requires further special investigation.

Twenty-four hours after administration, there was no hypertensive effect in the group of L-NAC, which is consistent with the fact that this drug has no hypertensive effect. On the contrary, the agents having undeniable hypertensive properties, such as HS, dexamethasone and HyperHAES, maintain blood pressure at the level close to the normal levels 24 hours after administration. The action time can be assumed by the effect on the blood pressure level. At the end of the experiment on the 6th day, low blood pressure was observed in the groups in which hyperosmolar solutions, dexamethasone and L-NAC had been used (Table 1). These differences can not be explained by using the data available in the study.

The ALI model induced by administration of acidin-pepsin into the trachea along with the supporting therapy by means of ceftriaxone resulted in 50% of deaths in the group. Using the above treatment regimens, including using liposomal aprotinin, failed to significantly change the mortality rate within 6 days of the experiment. The highest rate of the animals' survival was observed in the group with a conventional HS. On the 6th day, it was 83.3%. On 1st day, it was significantly higher than that without the experimental treatment and reached 91.7%.

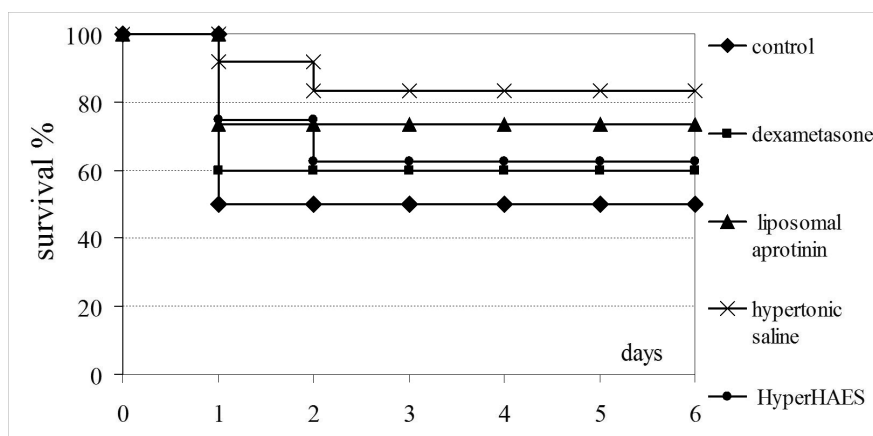


Figure 5. Animal survival rate in acute lung injury caused by aspiration of **acidin-pepsin** after using various types of therapy.

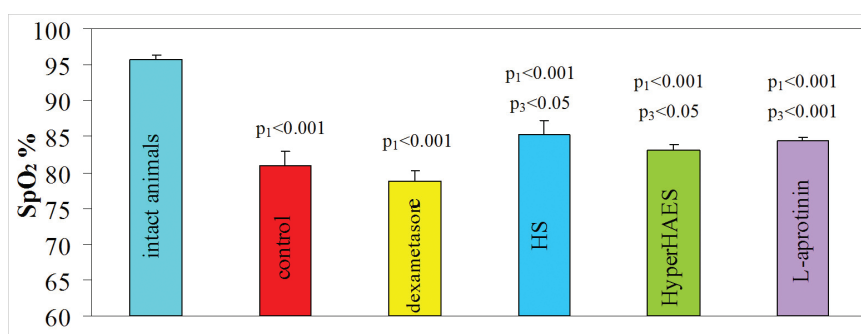


Figure 6. The changes in blood hemoglobin saturation in rats 1 hour after administration of the studied agents on the background of acute lung injury. Note: p₁ – significance of differences, calculated in relation to the group of intact animals. p₃ – in relation to control group 2 (**dexamethasone** 6 mg/kg, intravenously once+ **ceftriaxone** 200 mg/kg).

The animals which had received **dexamethasone** had a survival rate of 60%, those animals which had received liposomal **aprotinin** – 73.4% (Fig. 5). On the 1st day after administration of **HyperHAES** solution, the survival rate among the animals reached 75%, on the 2nd day – 62.5%.

One hour after the application, the therapy by means of **HS**, **HyperHAES** and liposomal **aprotinin** increased the SpO₂ level relative to the reference therapy based on a single administration of **dexamethasone** (Fig. 6). The survival rate and the SpO₂ level were the highest with the administration of **HS** – 85.3±1.9% – on the first day of the experiment. In other groups, an increase in hemoglobin saturation was also accompanied with an increase in the survival rate. After administration of **dexamethasone**, the SpO₂ level was the lowest among the reference regimens and reached 78.8±1.5%. When using **HyperHAES**, hemoglobin saturation reached 83.0±0.9%, when using liposomal **aprotinin** – 84.5±0.5%. Lack of significant differences from control group 1 makes it impossible to talk about the effectiveness of experimental therapy one hour after the application (Fig. 6).

Twenty-four hours later, the situation changed. Liposomal **aprotinin** kept the SpO₂ level at the same level as that one hour after administration – 83.5±2.1% (Fig. 7). In the groups of animals with administration of hyperosmolar solutions, by that time hemoglobin saturation was the

lowest (Fig. 7). After administration of **HS** and **HyperHAES**, the SpO₂ level was 78.3±2.6% and 78.6±0.9%, respectively. The rats which had received **dexamethasone** retained a higher level of SpO₂ – 83.2±1.2%. The recovery of the SpO₂ level in the control group reached 82.4±2.3%. Changes in oxygenation on the 1st day after applying the therapy reflect the causes of the survival pattern formation of the experimental animals at the same time (Fig. 7).

In the group where for the animals were given an antibiotic for 6 days, the SpO₂ level was 86.7±2.2%, in the group of **dexamethasone** – 82.6±2.1%. The rats that had received **HS** and **HyperHAES** as a treatment of on the 6th day had the SpO₂ level of 83.7±4.0% and 81.2±2.0%, respectively. Rats that had received liposomal **aprotinin** had saturation of 85.0±1.2% on the 6th day. For all the types of therapy, the SpO₂ level on the 6th days it was lower than that in healthy rats (Fig. 8).

One hour after aspiration of **acidin-pepsin** and intravenous administration of **HS**, the respiratory rate was 28.9% higher than that in the intact animals and 35.8% higher than that in control group 1 (Table 2). Twenty-four hours later, RR in the control group was high relative to the healthy animals and exceeded that by 24.8%. In the group with the administration of **HS**, 24 hours later the RR was also high, exceeding that in the intact animals

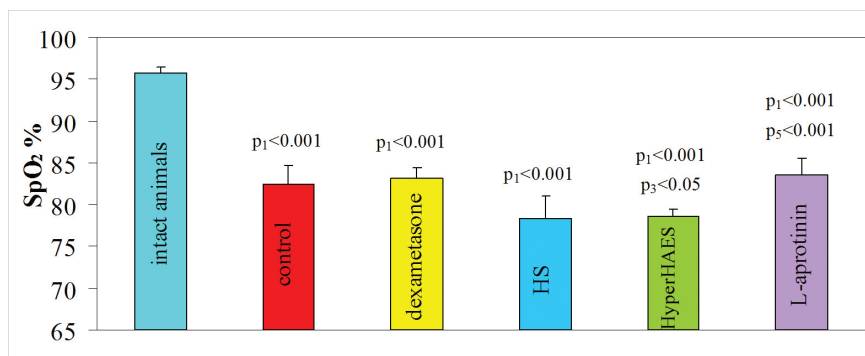


Figure 7. The changes in blood hemoglobin saturation in rats 24 hours after administration of the studied agents on the background of acute lung injury. Note: p₁ – significance of differences, calculated in relation to the group of intact animals. p₃ – in relation to control group 2 (dexamethasone 6 mg/kg, intravenously once + ceftriaxone 200 mg/kg). p₅ – in relation to the group with HyperHAES 4 ml/kg intravenously once + ceftriaxone 200 mg/kg.

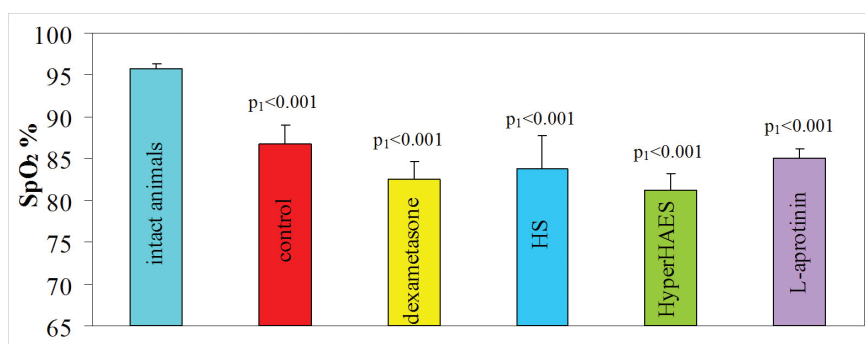


Figure 8. The changes in blood hemoglobin saturation in rats 6 days after administration of the studied agents on the background of acute lung injury. Note: p₁ – significance of differences, calculated in relation to the group of intact animals.

by 41.6%. Six days after the aspiration lung injury, the respiratory rate was high in the groups where the animals had received hypertonic solutions. In particular, 6 days after the administration of HS, RR was 34.8% higher than that in the healthy rats, and, in the group with the administration of HyperHAES, RR exceeded such in the group of the intact animals by 51.3%, in the control group – by 52.5 % and in the group with dexamethasone – by 84.8% (Table 2).

The role of dysfunction of the cardiovascular system in the general status and mortality of the experimental animals with acute lung injury induced by acidin-pepsin can be traced at an early stage, 1 hour after the application of the therapy. Thus, it is possible to observe reduction in the heart rate by 15%. Against this background, hypotension developed, systolic BP reduced by 31%, diastolic – by 31%. The hyperosmolar solutions and liposomal aprotinin used stabilize, in the first place, the heart rate (Table 2). The stimulating effect of hyperosmolar solutions on the level of systolic BP was also registered one hour after application of the therapy. In the same case, the reference therapy, which included the administration of dexamethasone, as well as the administration of liposomal aprotinin, had no corrective effect on the level of blood pressure (Table 2).

Twenty-four hours and then 6 days later, no significant changes in either heart rate or blood pressure were recorded, which would correspond to changes in the mortality rate in the studied groups. Thus, the recovery of the function of the cardiovascular system in surviving rats happened in a similar manner as in the previous model, with the use of acetone, which is due to a one-time therapy application (Table 2).

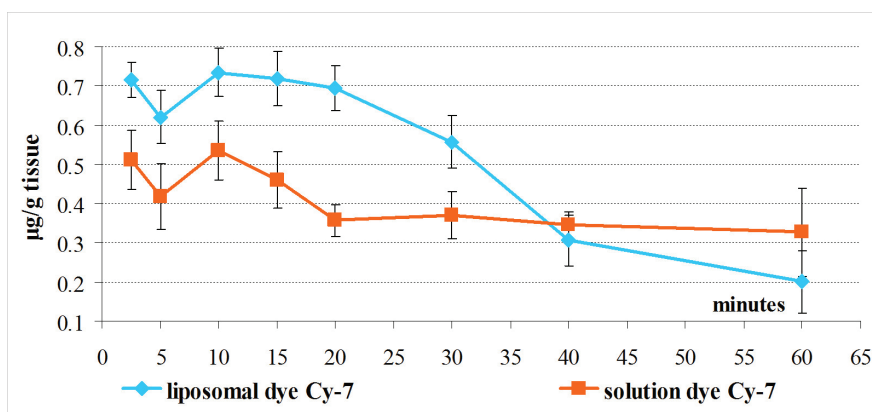
The experiment with fluorescent liposomes in mice with eviscerating the internal organs showed the following; Up to the 20th minute from the moment of intravenous injection, the liposomal and free dyes had a quite close concentration in blood, within the statistical error (Fig. 10). At the same time, the concentration of free dye in the lungs was 0.36±0.04 µg/g of tissue, liposomal dye – 0.69±0.06 µg/g, which was twice as much, and this was the largest difference in the accumulation of liposomal Cy-7 in lung tissue between the groups.

In the lungs and in blood, the concentration of the fluorescent dye, administered as a solution, did not change significantly until the 60th minute of observation (Fig. 9). At the same time, accumulation of the dye began in the liver starting with the 20th minute and lasted until the 60th minute, when it reached a maximum of 2.43±0.25 µg/g

Table 2. Changes in functional indicators in rats with acute lung injury caused by aspiration of **acidin-pepsin** after administration of reference therapeutic drugs ($M \pm m$, $n=10$).

Time	Indicators	Groups of experimental therapy				
		Control	Dexametasone	HS	HyperHAES	L-aprotinin
1 hour	RR 1/min	78.8±7.9	80.0±8.7	106.9±8.5 $p_{1,2}<0.05$	75.7±6.1 $p_4<0.05$	97.4±4.5 $p_5<0.05$
	HR 1/min	298.4±16.4 $p_1<0.05$	313.7±23.1	310.1± 25.1	380.5±17.8 $p_{2,3,4}<0.05$	325±15.3 $p_5<0.05$
	Systolic BP mm Hg.	93.5±6.5 $p_1<0.05$	103.4±13.1 $p_1<0.05$	124.1±11.6 $p_2<0.05$	121.3±9.8 $p_2<0.05$	108.0±4.6 $p_1<0.05$
	Diastolic BP mm Hg	67.5±6.8 $p_1<0.05$	72.3±11.3 $p_1<0.05$	77.0±8.0 $p_1<0.05$	87.2±9.9	80.3±4.8 $p_1<0.05$
24 hours	RR 1/min	103.7±6.5 $p_1<0.05$	97.0±15.9	117.7±4.4 $p_1<0.05$	102.5±22.5	112.5±18.5
	HR 1/min	364.3±19.8	328.0±24.0	369.0±19.1	347.5±39.5	294.6±22.7
	Systolic BP mm Hg.	122.9±6.1	146.7±19.1	149.0±8.5	111.5±6.5	111.5±12.6
	Diastolic BP mm Hg	91.6±5.0	96.0±17.2	105.3±13.3	87.8±20.2	75.5±15.1
6 days	RR 1/min	82.4±7.4	68.0±14.7	112.0±13.6 $p_1<0.05$	125.7±12.7 $p_{1,2,3}<0.05$	82.2±11.4
	HR 1/min	329.6±21.3	328.7±77.2	404.8±14.9 $p_2<0.05$	411.3±33.3	401.8±10.3 $p_2<0.05$
	Systolic BP mm Hg.	132.0±11.2	144.7±5.9	107.4±17.8	136.0±9.1	132.0±13.0
	Diastolic BP mm Hg	102.6±9.5	102.0±6.8	63.2±15.7 $p_{1,2}<0.05$	97.7±7.3	94.8±9.8

Note: p_1 – significance of differences, calculated in relation to the group of intact animals; p_2 – in relation to control group 1 (**acidin-pepsin** intratracheally 0.1 ml/kg); p_3 – in relation to control group 2 (**dexamethasone** 6 mg/kg, intravenously once + **ceftriaxone** 200 mg/kg); p_4 – in relation to the group with **hypertonic saline** intravenously once at a dose of 4 ml/kg + **ceftriaxone** 200 mg/kg; p_5 – in relation to the group with **HyperHAES** once at a dose of 4 ml/kg + **ceftriaxone** 200 mg/kg; **HS** – **hypertonic saline**; **RR** – respiratory rate; **HR** – heart rate; **BP** – blood pressure.

**Figure 9.** Accumulation dynamics of liposomal and dissolved dye **Cyanine-7** in the lung tissue of mice after intravenous administration.

of tissue (Fig. 11). A different process took place with liposomal **Cy-7**. Starting with the 20th minute, a decrease in the concentration of liposomal dye in the blood and lungs began, and a decrease in the **Cy-7** content in the liver stopped. The concentration of the dissolved dye in the lungs at the point of the 20th minute was 1.8 times higher than that in the blood. In the case of liposomes, the same concentration at the point of the 20th minute was 4 times higher in lung tissue. This may clearly indicate a more intense accumulation of liposomes in the lungs compared with the usual solution of the substance.

At the 40th minute, the concentrations of dissolved and liposomal **Cy-7** in the lungs were comparable (Fig. 9). In the lungs 40 minutes later, the concentration of the liposomal dye was 8.3 times higher than that in blood, while for the solution this difference was 1.75 (Fig. 10). Then, at the 60th minute, the concentration of liposomal **Cy-7** was 6.5 times higher than that in blood at the same time, and for the solution, the **Cy-7** concentration ratio in blood and lungs remained the same. Starting with the 40th minute, there began an intensive accumulation of liposomal **Cy-7** in the liver to reach 1.63 ± 0.2 µg/g of tissue, which was

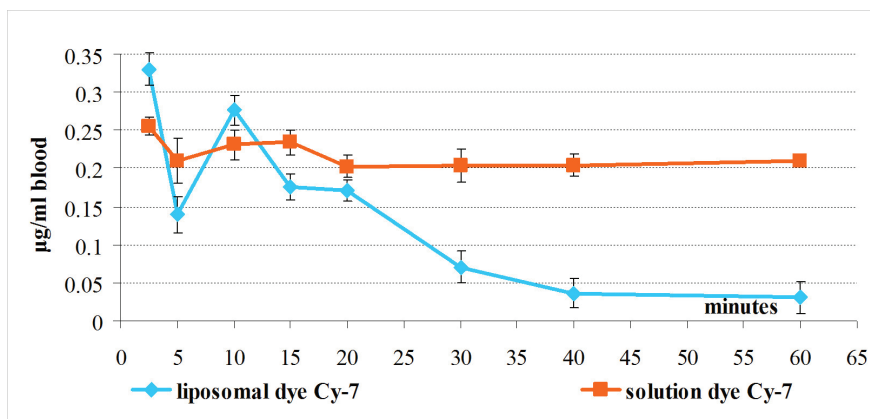


Figure 10. Accumulation dynamics of liposomal and dissolved dye Cyanine-7 in the blood of mice after intravenous administration.

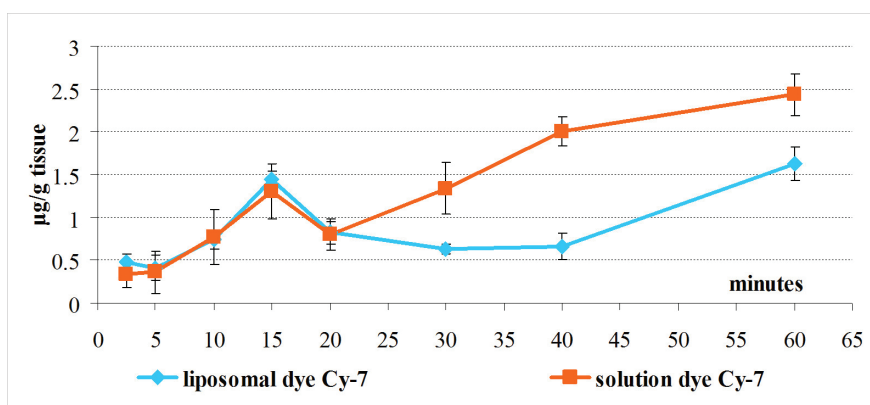


Figure 11. Accumulation dynamics of liposomal and dissolved dye Cyanine-7 in the liver tissue of mice after intravenous administration.

less than that for the dissolved form of the dye at the same time point (Fig. 9).

The dynamic pattern of the Cy-7 dye concentration in kidney tissue can be accounted for the similar processes. Liposomes accumulate in renal glomeruli (Kraft et al. 2014). The result is that within the first 30 minutes the concentration of the dye exceeded the liposomal dissolved dye concentration. A slow increase in the concentration of free and liposomal dyes in kidneys from the 40th to the 60th minutes after intravenous administration may be associated with the elimination of Cy-7 released from liposomes (Fig. 12).

Hyperosmolar liposomes with dexamethasone HLD compared with a combined therapy comprising HS and dexamethasone promoted a higher survival rate of animals with ALI caused by aspiration of acidin-pepsin. The survival rate after 6 days of the experiment in the group with HLD was 86.7%, in the group with HS+Dex – 66.7%, despite the fact that the similar doses of dexamethasone and HS were received by the animals in both groups. In comparison with the animals exposed to ALI induced by acidin-pepsin and treated with ceftriaxone, only HLD administration showed a statistically significant increase in the survival rate.

Looking at the dynamics of the therapeutic effect of HLD, the largest differences between the experimental and control groups were observed 4 hours after intraven-

ous administration of the therapeutic agents. Until then, in both groups the SpO₂ level had been recovering with equal intensity. Five minutes after aspiration, the saturation in the group treated with HLD decreased to 79.4±3.7%, while in the control group – to 76.9±3.4% (Fig. 13). At the point of the 2nd hour after the intravenous injection, saturation in the groups with HLD was 89.6±1.4%, in control – 89.0±1.4%. But by the 4th hour, in the group with HLD, the SpO₂ level increased, reaching 92.6±1.0%, while in the control it decreased to 86.5±1.7%. The opposite trend with the SpO₂ changes may be due to the fact that liposomal and free dosage forms of dexamethasone were used in the experiment. The liposomal form continued to correct respiratory disorders, while HS+Dex 4 hours later did not contribute to the growth of hemoglobin saturation. In the experimental group with HLD and in the group with HS+Dex, one day after administration, the saturation levels were 90 and 89%, respectively (Fig. 13).

A more prolonged positive effect of HLD on the respiratory function was confirmed by the various dynamics of the respiratory rate. In the group with HLD, during the development of ALI, the respiratory rate was suppressed from 85.3±7.0/min to 74.6±14.8/min. In the group with HS+Dex therapy, a decrease was more significant – from 65.4±6.3/min to 44.0±6.9/min (Fig. 14). But only in the latter case, it was possible to speak about the reliable oppression of RR. The graph of the change in RR shows

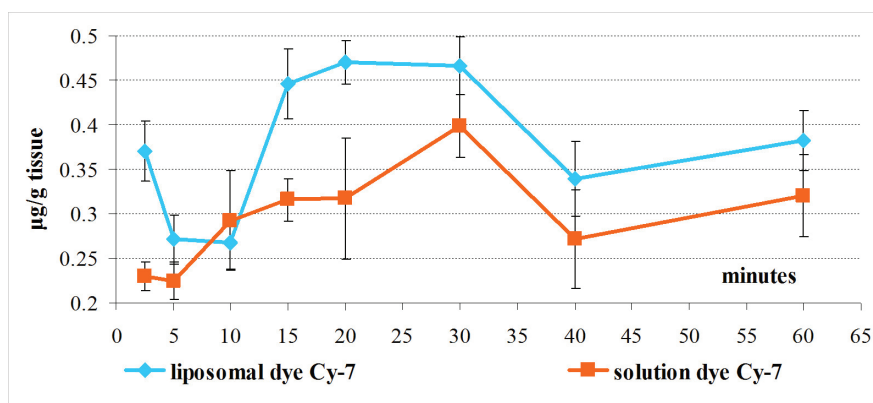


Figure 12. Accumulation dynamics of liposomal and dissolved dye *Cyanine-7* in kidney tissue of mice after intravenous administration.

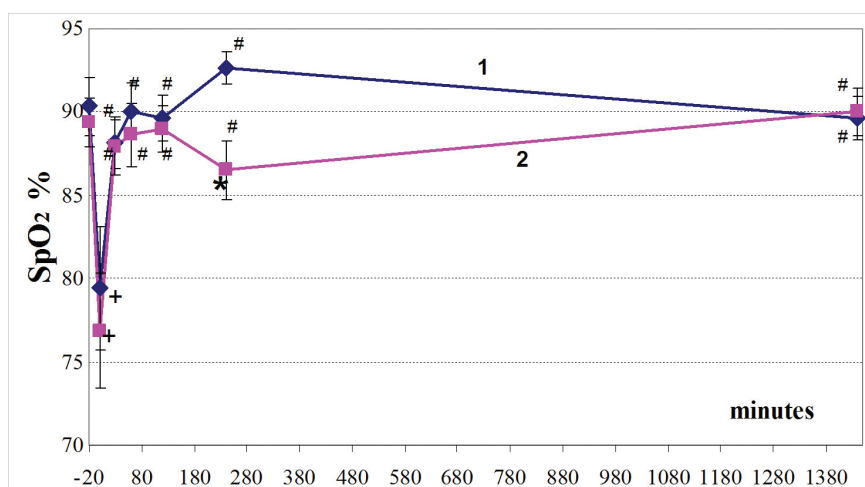


Figure 13. Hemoglobin saturation in rats with acute lung injury after using hyperosmolar liposomal *dexamethasone* (1) and *dexamethasone* in combination with *hypertonic saline* (2). Note: # – significance of differences calculated in relation to the value 5 minutes after the simulation of acute lung injury, at $p < 0.05$; * – significance of differences calculated between the control and experimental groups, at $p < 0.05$; + – significance of differences calculated in relation to the initial value, at $p < 0.05$.

that at the 4th hour in the HLD group there was a tendency for an increase in RR, i.e. recovery to the initial value, whereas in the group with the combination of *HS+Dex* there was a reverse trend, i.e. a decrease in RR, with a return to the value 5 minutes after aspiration of *acidin-pepsin* (Fig. 14).

Twenty-four hours later, the RR was restored to the baseline in both groups. Also it can be assumed that the combination of *HS+Dex* had a faster, but less prolonged stimulatory effect on the respiratory function. HLD led to an increase in RR by 30 minutes at a delayed point in time (Fig. 14). The therapeutic effects of HLD and *HS+Dex* when analysing the inspiratory and expiratory rates, as well as the expiratory volume, are similar. In both groups, there was a similar positive dynamics in the recovery of inspiratory rate, expiratory rate and expiratory volume. There was no difference in the speed of an onset of the effect with respect to these indicators. An important difference was the different levels of SpO_2 in the control and experimental group on the 6th day of observation. In the group with HLD,

the SpO_2 level was $88.6 \pm 1.3\%$, which was significantly higher than that 5 minutes after aspiration prior to administration of the therapeutic agent. In the group with treatment with *HS+Dex*, after 6 days the SpO_2 level was $86.0 \pm 2.0\%$ and had no statistically significant differences from the SpO_2 level which was fixed 5 minutes after aspiration of *acidin-pepsin*. These data may indicate a significant disruption of the respiratory function in the group treated with the reference therapy and, therefore, its lower effectiveness.

Functional diagnostics of the cardiovascular system demonstrated the effectiveness of HLD compared to the therapy with *HS+Dex*. HLD corrected the heart rate in a more pronounced way. As a result of aspiration, the heart rate was depressed. The heart rate in both groups reduced on average by 50% of the original (Fig. 15). In the healthy animals before aspiration of *acidin-pepsin* the heart rate was about 215/min for both groups of animals. After aspiration, administering HLD caused 30 minutes later an increase in the heart rate from 123 ± 16 /min to 193 ± 16 /min (Fig. 15). Further growth continued, and an hour later

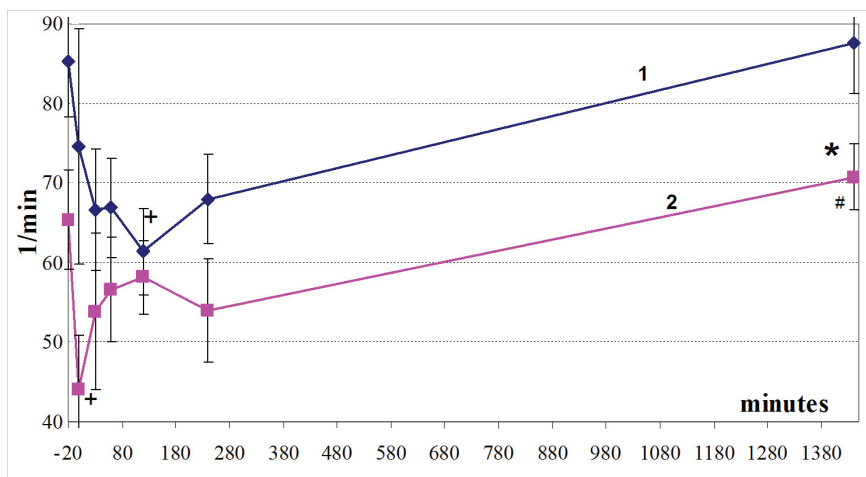


Figure 14. Respiratory rate in rats with acute lung injury after using hyperosmolar liposomal dexamethasone (1) and dexamethasone in combination with hypertonic saline (2). Note: # – significance of differences calculated in relation to the value 5 minutes after the simulation of acute lung injury at $p < 0.05$; * – significance of differences is calculated between the control and experimental groups at $p < 0.05$; + – significance of differences calculated in relation to the initial value at $p < 0.05$.

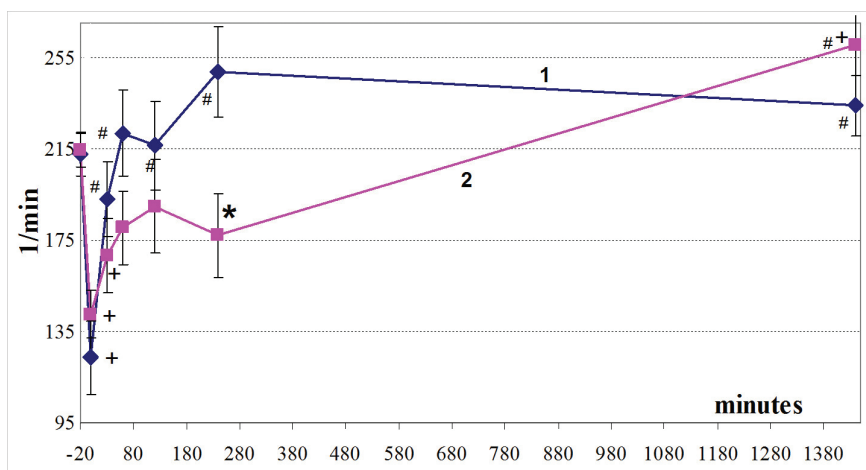


Figure 15. Heart rate in rats with acute lung injury after using hyperosmolar liposomal dexamethasone (1) and dexamethasone in combination with hypertonic saline (2). Note: # – significance of differences calculated in relation to the value 5 minutes after the simulation of acute lung injury at $p < 0.05$; * – significance of differences calculated between the control and experimental groups at $p < 0.05$; + – significance of differences calculated in relation to the initial value at $p < 0.05$.

the heart rate was 221.9 ± 18.8 /min. Two, four and twenty-four hours after administration of HLD, the heart rates were 216 ± 19.5 , 248.8 ± 19.8 and 233.9 ± 13.2 /min, respectively.

In the control group 30 minutes later, the heart rate, though had increased from 142.5 ± 10.4 /min to 168.2 ± 16.3 /min, remained significantly lower than the initial value. In the control group 1 hour, 2 and 4 hours later, the heart rates in rats were 180.2 ± 16.2 , 189.7 ± 20.5 and 176.9 ± 18.0 /min, respectively (Fig. 15). At those time points, the heart rate was not significantly higher than that 5 minutes after aspiration of acidin-pepsin.

The effect of HLD therapy and HS+Dex therapy on systolic blood pressure was similar (Fig. 16).

One may notice the different pattern of the dynamics of diastolic BP 4 hours after the intravenous administration

of the therapeutic agents. While in the experimental group of rats with HLD, diastolic BP continued to grow to reach 119.7 ± 6.3 mm Hg, in the control group, it decreased to 97.1 ± 7.1 mm Hg (Fig. 17). In this case, again one can assume a longer effect of HLD compared with that of HS+Dex.

HyperHAES and HS affected the degree of pulmonary edema in different ways. On the first day, HyperHAES was more effective, as it reduced the level of pulmonary edema compared to the animals without administration of the experimental therapeutic agents (Table 3). A similar effect was observed after administration of dexamethasone (control 2). HS did not affect significantly on the LB ratio.

On the 6th day after the administration of hyperosmolar solutions, it was possible to compare the LB ratio only with that in the intact animals. After treatment with HS and HyperHAES solution and the reference treatment

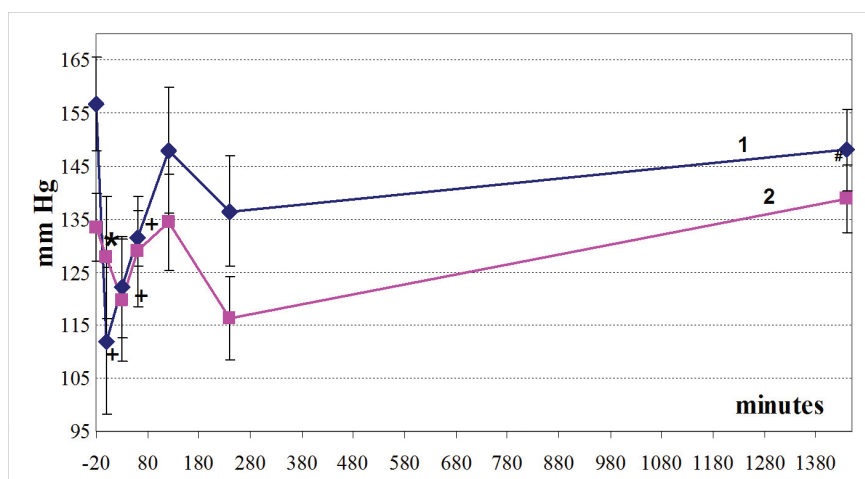


Figure 16. Systolic blood pressure in rats with acute lung injury after using hyperosmolar liposomal **dexamethasone** (1) and **dexamethasone** in combination with **hypertonic saline** (2). **Note:** # – significance of differences calculated in relation to the value 5 minutes after the simulation of acute lung injury at $p < 0.05$; * – significance of differences calculated between the control and experimental groups at $p < 0.05$; + – significance of differences calculated in relation to the initial value at $p < 0.05$.

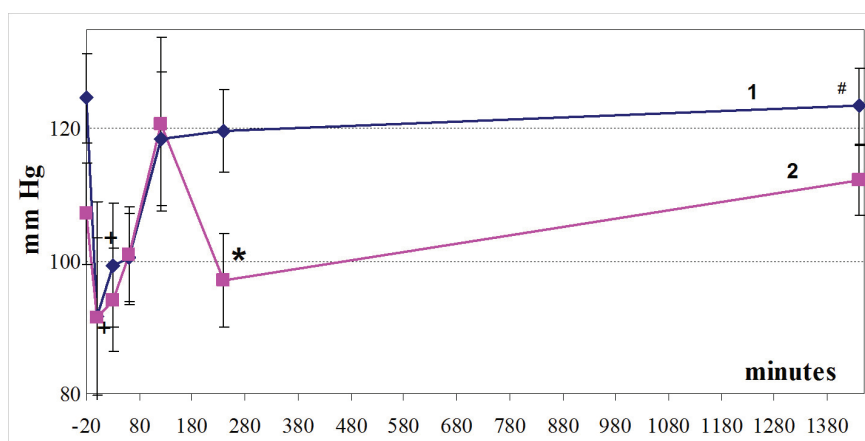


Figure 17. Diastolic blood pressure in rats with acute lung injury after using hyperosmolar liposomal **dexamethasone** (1) and **dexamethasone** in combination with **hypertonic saline** (2). **Note:** # – significance of differences calculated in relation to the value 5 minutes after the simulation of acute lung injury at $p < 0.05$; * – significance of differences calculated between the control and experimental groups at $p < 0.05$; + – significance of differences calculated in relation to the initial value at $p < 0.05$.

with **dexamethasone**, an increase in pulmonary edema was observed by the 6th day of the experiment. The LB ratio was significantly higher than that in the intact animals (Table 3). Thus, it is difficult to talk about the advantage of any one hyperosmolar solution for infusions, as the most effective one for eliminating pulmonary edema in this model. **HyperHAES** and **HS** had no antiedematous effect during aspiration of **acetone** by the experimental animals. Compared with other experimental groups L-NAC was the best in preventing the development of pulmonary edema, since 24 hours after administration of L-NAC, the LB ratio was similar to that in other experimental groups and remained such until the 6th day (Table 3). Here, one can talk of the protective effect of L-NAC in this form of the aspiration lung injury.

Acute lung injury caused by intratracheal administration of **acidin-pepsin** solution caused not as a pronounced

increase in the LB ratio as when using **acetone**. The LB ratio in control group was almost the same both 24 hours and 6 days later (Table 4). The use of **dexamethasone** and hyperosmolar solutions led to an increase in pulmonary edema, rather than a decrease in it, although those values did not have statistically significant differences relative to the control group. The combination therapy of **dexamethasone** and **HS** used on this model reduced the degree of pulmonary edema very effectively. The LB ratio with this therapy did not differ from that of normal lungs and was smaller than with applying a monotherapy with hyperosmolar solutions and **dexamethasone** separately. The LB ratio on the 6th day in the group with **HS+Dex** was comparable to that in the healthy animals, which also confirms the effectiveness of this combination for the treatment of pulmonary edema (Table 4). Liposomal forms of **aprotinin** and **dexamethasone** also performed

Table 3. Rats' lung-body ratio in acute lung injury caused by aspiration of acetone after using experimental therapeutic agents (M±m, n=10).

Time	Groups of experimental therapy					
	Control	Dexametasone	HS	HyperHAES	L-NAC	Intact animals
24 hours	13.4±0.9 p ₁ <0.001	8.6±1.1 p ₂ <0.05	11.2±0.7 p ₁ <0.001	7.63±0.6 p ₂ <0.01 p ₄ <0.05	8.5±0.6 p _{1,4} <0.05 p ₂ <0.01	7.0±0.2
6 days	–	11.1±0.5 p ₁ <0.001	11.5±0.7 p ₁ <0.001	10.5±0.4 p ₁ <0.001	8.9±0.4 p ₁ <0.01 p _{3,4,5} <0.05	

Note: p₁ – significance of differences, calculated in relation to the group of intact animals; p₂ – in relation to control group 1 (acetone intratracheally); p₃ – in relation to the group with dexamethasone once intravenously; p₄ – in relation to the group with hypertonic saline once intravenously; p₅ – in relation to the group with HyperHAES once intravenously; HS – hypertonic saline; L-NAC – liposomal NAC.

Table 4. Rats' lung-body ratio in acute lung injury caused by aspiration of acidin-pepsin after using experimental therapeutic agents (M±m, n=10).

Time	Groups of experimental therapy							Intact animals
	Control	Dexa-metasone	HS	Hyper HAES	L-aprotinin	HLD	HS+Dex	
24 hours	9.7±0.5 p ₁ <0.01	11.0±2.0 p ₁ <0.05	11.9±3.3 p ₁ <0.05	12.3±0.6 p ₁ <0.001 p ₂ <0.05	8.2±0.6 p ₁ <0.05 p ₅ <0.01	5.3±0.3 p _{1,4} <0.001 p _{2,3} <0.05 p _{3,6} <0.01	6.4±1.1 p ₂ <0.05 p ₃ <0.01	7.0±0.2
6 days	9.8±1.0 p ₁ <0.05	11.4±1.6 p ₁ <0.01	12.3±0.9 p ₁ <0.001	14.7±0.8 p ₁ <0.001 p ₂ <0.05	7.3±1.0 p _{4,5} <0.01	7.6±1.5 p _{4,5} <0.05	7.5±1.4 p ₄ <0.05 p ₃ <0.01	

Note: p₁ – significance of differences, calculated in relation to the group of intact animals; p₂ – in relation to control group 1 (acidin-pepsin intratracheally); p₃ – in relation to the group with dexamethasone once intravenously; p₄ – in relation to the group with hypertonic saline once intravenously; p₅ – in relation to the group with HyperHAES once intravenously; p₆ – in relation to the group with liposomal aprotinin once intravenously; HS – hypertonic saline; HLD – hyperosmolar liposomal dexamethasone; HS+Dex – dexamethasone in combination with hypertonic (7.5%) sodium chloride solution.

well on this model. L-aprotinin 24 hours later reduced the degree of pulmonary edema in a more pronounced way than HyperHAES. In the group with HLD 24 hours later, the LB ratio was the lowest. Six days later, the lungs of the animals after the use of liposomal forms of aprotinin and dexamethasone had the LB ratio at the same level as in intact rats.

Thus, on the 6th day, the LB ratio in the animals treated with liposomal forms approached the value of healthy lungs in two ALI models.

Discussion

During 6 days of the observation after aspiration of acetone, the lowest mortality rate was observed in rats that had received an intravenous injection of liposomal NAC. However, the greatest difference was in relation to the group in which no experimental therapy had been used, which means that all the proposed schemes are a way to reduce the mortality rate in case of aspiration of acetone.

The SpO₂ level recovered in all the animals that had survived until the 6th day to a level that did not differ statistically, whereas the level remained lower than that observed in the healthy animals. This suggests that the recovery of the respiratory function had a similar positive trend, regardless of a type of the drug substance. Since the RR in rats with ALI fluctuated unevenly, its changes cannot be attributed to the causes of mortality and a decrease of blood oxygen saturation in this study. The fact that on the 6th day after the aspiration of acidin-pepsin the blood oxygenation in the control group with ceftriaxone-based supporting therapy rose to 86.7±2.2% can mean that the recovery of the respiratory function 6 days after the start of the experiment hardly depends on a single injection of the tested drugs, and the main influence was made by the daily administration of ceftriaxone, preventing the development of purulent aspiration pneumonia.

The use of liposomal aprotinin can be considered an effective remedy for ALI. Perhaps high efficiency of liposomal aprotinin is associated with its antiproteolytic

properties and a liposomal form, which ensured survival within the ALI acute period up to 73.4% and a low LB ratio on the 1st and 6th days, which indicates effective prevention of pulmonary edema. When comparing the pathological manifestations of the two models used, it can be said that **acidin-pepsin** exerted its damaging effect more slowly, which was due to the fact that unlike **acetone**, **acidin-pepsin** acted as a result of proteolysis of the pulmonary tissue protein structures. Proteolysis had already led to the destruction of the lung tissue. The feature of the pathogenesis of the ALI model showed in the effectiveness of the applied therapy. The reference therapy with **dexamethasone** would not lead to significant reductions in the manifestations of ALI, while the effect of liposomal **aprotinin** would lead to a positive change not on the 1st, but on the 6th day, which confirms the effectiveness of liposomal **aprotinin** in this model.

Taking into account the survival rate of the animals by the 6th day of the experiment, it can be concluded that the liposomal form of **dexamethasone** in the **hypertonic NaCl solution** proved to be an effective means for controlling aspiration of **acidin-pepsin**. HLD ensured high pharmacological efficacy in ALI. It may have been due to the peculiar release of **dexamethasone** from liposomes and an additional osmotic effect of 7.5% NaCl. In the form of monotherapy, **HS** also ensured a high survival rate of the animals in this model of ALI. A marked therapeutic effects of HLD can be due, first, to the peculiarities of drug release, which, like other membrane structures (erythrocytes) respond to changes in the environmental osmolarity. In this case, with intravenous administration, the environment around liposomes became isotonic, while the liposome content - was hypertonic. Under the influence of diffusion forces, **dexamethasone** along with **hypertonic saline** could be released from liposomes into blood (Monteiro et al. 2015). However, to prove this, more research is needed. Secondly, the reason of high efficiency of HLD may be related to the **lecithin** which is part of liposomes. **Phosphatidylcholine**, as the main component of liposome vesicles, exhibit surface active properties and, thus, can prevent the collapse of alveoli in ALI (Rugonyi et al. 2008).

The specific mechanisms of the positive effect of **phosphatidylcholine** on lung tissue remain unclear. However, a number of studies make the picture more obvious. In particular, Gwinn et al. (2011) showed that surface-active properties of **phosphatidylcholine** promote apoptosis of neutrophils and, as a consequence, reduce the severity of pneumonia. There is also evidence that indicate a positive effect of liposomal lipids on ventilation, a histopathological picture and a surfactant levels in laboratory animals with ALI (Rugonyi et al. 2008). Liposomal lipids can exert an antioxidant effect, as they take part in oxidation processes, protecting cell membranes from oxidation (Meng and Xu 2015).

HLD therapy turned out to be the most effective for preventing the development of pulmonary edema. The

advantage of HLD therapy over the reference **HS+Dex**-based therapy was observed on the 1st and 6th days of the experiment. Thus, liposomal dosage forms used for treatment of **acidin-pepsin** aspiration model showed a higher therapeutic efficacy compared to that in the other regimens. Hypertonic solutions affected a degree of pulmonary edema in both ALI models in a way which was comparable to the treatment with **dexamethasone** at the stated dose.

Looking at the kinetics of the in vivo distribution of liposomes, it can be noted that it depends on the expected size of liposomes used and is consistent with some literature data (Li et al. 2017), describing the dependence of the biodistribution of nanoparticles injected intravenously on their size. Liposomes used in the study were large enough, whose vesicle size ranged within 350 nm. Liposomes were simple and contained a membrane of phospholipids, and, therefore, could quickly undergo biodegradation under the action of enzyme systems, blood and tissues (Meng and Xu 2015). Considering that the concentration of the free dye in blood within one hour after the administration did not significantly fluctuated, it can be concluded that liposomes, as carriers of a drug substance, better than a dissolved form ensure accumulation of the drug in internal organs, primarily lungs. If the accumulation of the liposomal form of **Cy-7** increased in the lungs after intravenous administration, simultaneously its content reduced in the liver and kidneys. The liver showed itself as a primary concentrator of the dye administered intravenously, regardless of its form. The liver accumulated the solute more actively, while the liposomal substance in liposomal vesicles of about 350 nm accumulated longer in the lungs. Including the substance into liposomes was aimed at creating its high concentration in the lung, as a result of intravenous administration. The goal was achieved by using liposomes of the appropriate size. By means of liposomes, the substance after intravenous administration could contact more with lung tissue, which was a target for the therapy in the current study, and less with the liver, which is the main organ for metabolizing and inactivating the drug.

Conclusion

The experimental results showed good perspectives of parenteral use of liposomal drug forms containing **N-acetylcysteine**, **aprotinin**, **dexamethasone**, as well as hyperosmolar solutions based on sodium chloride, as means to correct the main pathological manifestations of acute lung injury.

The identified features of biodistribution of liposomes with a diameter of about 350 nm justify the intravenous administration of liposomal forms to increase the accumulation of drugs in the lungs when treating ALI.

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