Effect of Different Dosages of Dexamethasone Therapy on Lung Function and Inflammation in an Early Phase of Acute Respiratory Distress Syndrome Model

P. MIKOLKA[1,2], P. KOSUTOVA[1,2], M. KOLOMAZNIK[1,2], J. TOPERCEROVA[2], J. KOPINCOVA[2], A. CALKOVSKA[1,2], D. MOKRA[1,2]

[1]Biomedical Center Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic, [2]Department of Physiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic

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Summary

Inflammation associated with acute respiratory distress syndrome (ARDS) can damage the alveolar epithelium and surfactant and worsen the respiratory failure. Glucocorticoids (GC) appear to be a rational therapeutic approach, but the effect is still unclear, especially for early administration and low-dose. In this study we compared two low doses of dexamethasone in early phase of surfactant-depleted model of acute respiratory distress syndrome (ARDS). In the study, lung-lavaged New Zealand rabbits with respiratory failure (PaO_2<26.7 kPa in FiO_2 1.0) were treated with intravenous dexamethasone (DEX): 0.5 mg/kg (DEX-0.5) and 1.0 mg/kg (DEX-1.0), or were untreated (ARDS). Animals without ARDS served as controls. Respiratory parameters, lung edema, leukocyte shifts, markers of inflammation and oxidative damage in the plasma and lung were evaluated. Both doses of DEX improved the lung function vs. untreated animals. DEX-1.0 had faster onset with significant improvement in gas exchange and ventilation efficiency vs. DEX-0.5. DEX-1.0 showed a trend to reduce lung neutrophils, local oxidative damage, and levels of TNFα, IL-6, IL-8 more effectively than DEX-0.5 vs. ARDS group. Both dosages of dexamethasone significantly improved the lung function and suppressed inflammation in early phase ARDS, while some additional enhancement was observed for higher dose (1 mg/kg) of DEX.

Key words

Acute respiratory distress syndrome • Glucocorticoids • Dexamethasone • Lung function • Inflammation

Corresponding author

D. Mokra, Department of Physiology and Biomedical Center Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Mala Hora 4C, SK-03601 Martin, Slovak Republic. E-mail: daniela.mokra@uniba.sk

Introduction

Acute respiratory distress syndrome (ARDS) is still a serious life-threatening condition. Despite the favorable impact of the lung-protective ventilation strategy demonstrated in the ARDSnet study, the incidence and overall mortality of ARDS have not changed substantially (Villar *et al.* 2014). ARDS involves acute diffuse, inflammatory lung injury leading to increased pulmonary vascular permeability, and loss of aerated lung tissue. The clinical hallmarks are hypoxemia and bilateral radiographic opacities, associated with increased physiological dead space and decreased lung compliance (Ranieri *et al.* 2012). Increase in activated neutrophils is a critical final pathway of the lung injury during ARDS resulting in deterioration of alveolar-capillary membrane and alveolar epithelium by the release of toxic mediators (Zemans *et al.* 2009, Matthay and Zemans 2011). The epithelial injury leads to surfactant alterations and together with edema formation, ventilation-perfusion mismatch and inflammation reduces the lung compliance and hypoxemia and further deteriorates the lung function (Verbrugge *et al.* 1997, Pierrakos *et al.* 2012).

Fax +420 241 062 164, e-mail: physres@fgu.cas.cz, www.biomed.cas.cz/physiolres

Therapeutic protocol for ARDS is almost based on the lung-protective ventilation and fluid-conservative management. A large number of pharmacologic therapies, such as glucocorticoids (GC), surfactants, inhaled nitric oxide, antioxidants, protease inhibitors, and other anti-inflammatory drugs have been tested in the ARDS treatment, but without clear evidence of positive effect (Cepkova and Matthay 2006). GC have appeared to be perspective due to their multiple anti-inflammatory, anti-edematous and pulmonary vasodilator actions (Czock *et al.* 2005, Coutinho and Chapman 2011), particularly in systemic administration (Peter *et al.* 2008). Despite presumed therapeutic potential, use of GC in patients with ARDS led to controversial results (Meduri *et al.* 2007, Tang *et al.* 2009, Ruan *et al.* 2014). However, administration of dexamethasone in our previous experimental studies was of benefit and become the basis for this study. Dexamethasone positively influenced the lung functions and attenuated inflammation (Mokra *et al.* 2007, Kosutova *et al.* 2016). There is also increasing evidence that lower dose of GC administered earlier after developing ARDS could be beneficial (Meduri *et al.* 2007, Narute *et al.* 2017, Yang *et al.* 2017). However, due to heterogeneous nature of ARDS the appropriate dosing regimens, timing and duration of GC therapy as well as reasonability for their use are still discussed.

We hypothesized that low dose of dexamethasone in ARDS would decrease the inflammatory response and thereby alleviate the lung injury. Therefore, the purpose of this study was to determine the effects of two different low bolus doses of dexamethasone administered systemically on the lung function and the leukocyte and inflammatory profiles in an acute phase of experimental ARDS.

Methods

Animal instrumentation

This study was approved by the National Veterinary Board of Slovakia and the local Ethical Committee of Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava. Adult New Zealand white rabbits of both genders with a body weight (b.w.) of 2.8±0.3 kg were instrumented in accordance with previous study (Kosutova *et al.* 2017). After initial anaesthesia with intramuscular tiletamine and zolazepam (15 mg/kg b.w.; Zoletil, Virbac, France) and xylazine (5 mg/kg b.w.; Xylariem, Riemser, Germany), animals were sedated by i.v. infusion of anesthetics (tiletamine and zolazepam, 10 mg/kg/h). Subsequently, the animals were mechanically ventilated (Aura V, Chirana, Slovakia) with positive end-expiratory pressure (PEEP) of 0.5 kPa, tidal volume (V_T)<6 ml/kg, time of inspiration (Ti) 50 %, respiratory rate (RR) of 40 breaths per minute (bpm), and FiO_2 of 1.0 throughout the experiment. Finally, 4 h after the treatment, the animals were sacrificed by an overdose of anesthetics. Electrocardiographic monitoring using subcutaneous electrodes and invasive arterial pressure monitoring were carried out continuously using multi-channel recorder PowerLab 8/30 (AD Instruments, Germany). Gas exchange and parameters of acid-base balance were measured from arterial blood samples using blood gas analysis (RapidLab TM^{348}, Bayer Diagnostics, Germany). Ventilation parameters were measured by in-build sensors and software of ventilator Aura V. The following parameters were calculated: P/F = calculated as a ratio between arterial oxygen partial pressure (PaO_2) and fraction of inspired oxygen (FiO_2); oxygenation index (OI) = (Mean airway pressure x FiO_2) /PaO_2; ventilation efficiency index (VEI) = 3800 / [(PIP – PEEP) x frequency x $PaCO_2$]; and alveolar–arterial gradient (AaG) = [FiO_2 (P_{atm} – PH_2O) – $PaCO_2$ /0.8] – PaO_2, where P_{atm} is barometric pressure and PH_2O is pressure of water vapor.

Experimental model of ARDS

After 15 min of stabilization period, the respiratory parameters and blood gases were recorded (basal values, BV). According to modified protocol by authors, the lung injury was induced by repetitive lung lavage with a saline (30 ml/kg b.w., 37 °C) instilled into the endotracheal cannula in the semi-upright right and left lateral positions of the animal and immediately suctioned by a suction device. The lavages were repeated with stabilization periods of 2 min ventilation in between, until PaO_2 in the arterial blood decreased to <26.7 kPa in FiO_2 1.0 and lung compliance decreased of <30 % of the initial value in two measurements at 5 and 15 min after the last lavage, which equals moderate ARDS. At this point the respiratory parameters and blood gases were recorded again (Model). During the lavage protocol animals were ventilated using unchanged settings (V_T<6 ml/kg, PEEP 0.5 kPa, Ti 50 %, RR 40 bpm and FiO_2 1.0).

Treatment protocol

After the criteria of lung injury were full-filled, the animals were assigned randomly to the following

three groups (n=7 for each group): (i) ARDS group, without treatment; (ii) DEX-0.5 group, ARDS with 0.5 mg/kg dexamethasone i.v.; (iii) DEX-1.0 group, ARDS with 1.0 mg/kg dexamethasone i.v.; dexamethasone natrium phosphate (Dexamed sol inj, 8 mg/2 ml, Medopharm, Czech Republic). One group of animals served as healthy, ventilated and non-treated controls (Control, n=7). All animals were oxygen-ventilated (V_T<6 ml/kg, PEEP 0.5 kPa, Ti 50 %, RR 40 bpm and FiO_2 1.0) for additional 4 h. Post-treatment physiological data such as blood gases, acid-base balance and respiratory parameters were recorded at 30 min, 1, 2, 3 and 4 h after the therapy (Th).

Total and differential leukocytes count in arterial blood and BALF

After 4 h after the treatment and sacrificing the animal, lungs and trachea were excised. The left lung was lavaged with saline (3-times, 10 ml/kg b.w.) to obtain a bronchoalveolar lavage fluid (BALF). Total leukocyte count was determined microscopically in a counting chamber after staining by Türck. The differential cell counts in the BALF and arterial blood were estimated microscopically after staining by May-Grünwald/Giemsa-Romanowski.

Post-mortem analyses

Levels of cytokines, apoptotic marker and oxidative modifications were determined in plasma and in 10 % (weight/volume) lung homogenate. Oxidative modification was determined using kits: OxiSelect^{TM} Nitrotyrosine ELISA Kit (Cell Biolabs, Inc., USA) for protein oxidation expressed in 3-nitrotyrosine nanomolar concentration (nM 3NT); OxiSelect^{TM} TBARS Assay Kit (Cell Biolabs Inc., USA) for lipid oxidation expressed as malondialdehyde in micromolar concentration (μM MDA). Levels in pg/ml of TNFα, IL-6, and IL-8 were quantified using rabbit-specific ELISA kits (Cloud-Clone Corp., USA) and the apoptotic marker caspase-3 using rabbit-specific ELISA kit (Cusabio Biotech Co Ltd, China) according to the manufacturers' instructions.

Strips of the right lung lobe were weighed before and after drying in an oven at 60 °C for 48 h to calculate the wet-to-dry (W/D) lung weight ratio, extent of the lung edema. Total protein content in the BALF was determined by Bradford colorimetric method and were expressed in μg/ml.

Statistical analysis

Analysis of data was performed using statistical software Graph Pad Prism 6.01 (USA). Two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test for dynamic changes parameters and Kruskal-Wallis non-parametric test for groups comparison were used. A p<0.05 was considered for statistically significant. The results are presented as mean ± SEM.

Results

The data from 28 rabbits of both genders were used for the analysis. At the beginning of experiments, there were no significant differences in the entry parameters (body weights, gender rate) and the initial values of respiratory parameters among the all groups (for all p>0.05). Induction of ARDS affected the lung function parameters compared to the baseline values (time sequence Model vs. BV; p<0.001), however in the observed time point for Model, there were no significant differences in these parameters between all ARDS groups (ARDS vs. DEX-0.5 vs. DEX-1.0; for all p>0.05).

Lung function parameters

Induction of lung injury caused a significant deterioration in the lung function parameters. All observed respiratory parameters (PaO_2/FiO_2, OI, VEI, AaG, MAP, C_{dyn}, Raw) had been severely altered after the lavage compared to the controls (for all p<0.001) and this trend persisted till the end of the experiment (Fig. 1, Table 1).

Both doses of dexamethasone significantly improved P/F ratio, OI and AaG compared to ARDS, while the higher one (DEX-1.0) showed rapid onset (at 30 min Th p<0.05) and long term effect (at 4 h Th p<0.001) (Fig. 1). Other lung function parameters were also improved after DEX therapy (Table 1). Only DEX-1.0 improved VEI vs. ARDS at 3 and 4 h Th (p<0.05). Superior effect for DEX-1.0 towards DEX-0.5 was observed in P/F ratio and AaG (DEX-1.0 vs. DEX-0.5, p<0.05) (Fig. 1A, 1D).

Leukocytes in the blood and cells in the BALF

In the blood, total leukocytes count in the untreated ARDS animals significantly decreased vs. controls (p<0.05) what reflected a decrease in neutrophils (p<0.01) and monocytes (p<0.05). Both DEX therapies significantly elevated the circulating neutrophils (p<0.05

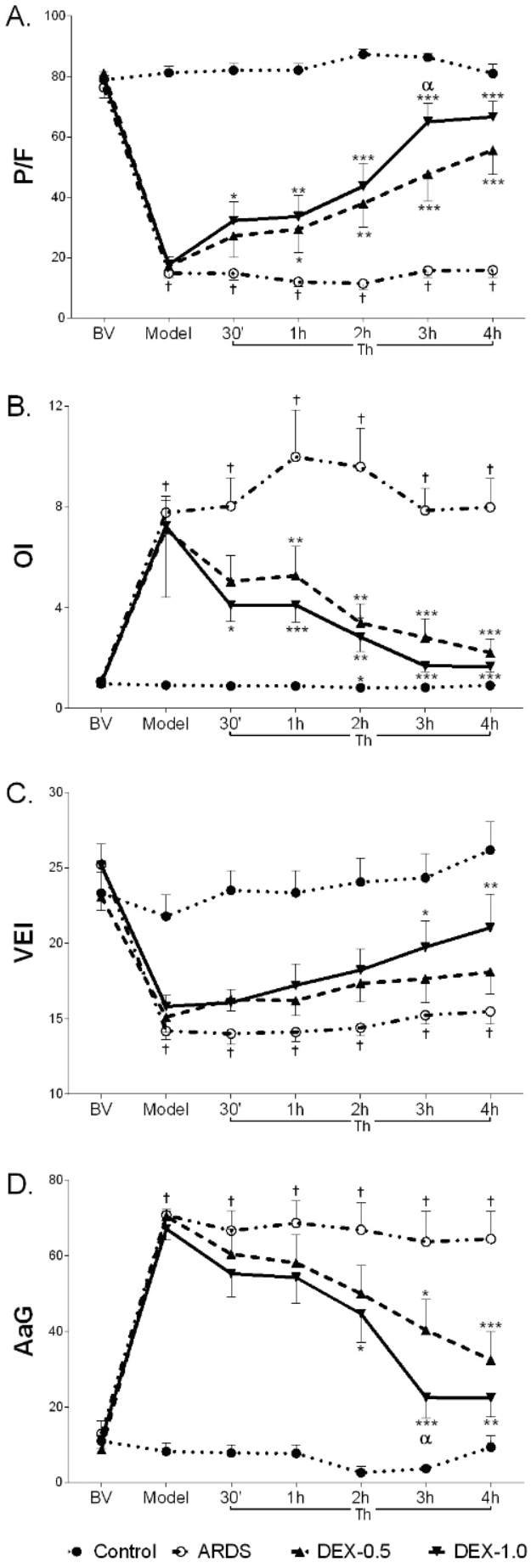

Fig. 1. Respiratory parameters: (**A**) the ratio of arterial oxygen partial pressure to fraction of inspired oxygen (P/F), (**B**) oxygenation index (OI), (**C**) ventilation efficiency index (VEI), (**D**) alveolar-arterial gradient (AaG) before (basal value, BV) and in ARDS condition (Model) and during 4 h after the therapy (Th) in Control group, ARDS untreated group, and ARDS groups treated with 0.5 mg/kg of dexamethasone (DEX-0.5) or 1.0 mg/kg of dexamethasone (DEX-1.0). Data are presented as means ± SEM. Statistical comparisons: for ARDS vs. Control † $p<0.001$; for DEX-0.5 & DEX-1.0 vs. ARDS * $p<0.05$, ** $p<0.01$, *** $p<0.001$; and for DEX-1.0 vs. DEX-0.5 $^{\alpha}$ $p<0.05$.

vs. ARDS), for DEX-1.0 also monocytes ($p<0.05$). In opposite, percentage of lymphocytes increased in ARDS group ($p<0.01$ vs. Control) and decreased after the DEX therapies ($p<0.05$ vs. ARDS group) (Table 2).

Local lung injury was linked with an influx of leukocytes into BALF with increase of total count ($p<0.001$), neutrophils ($p<0.001$) and eosinophils ($p<0.05$) and decrease of monocytes ($p<0.001$) in the ARDS group vs. Control. Both DEX therapies reduced total leukocytes count, with bigger impact for DEX-1.0 ($p<0.01$ vs. ARDS). Only DEX-1.0 significantly increased monocytes and decreased neutrophils leak into the lung (both $p<0.05$ vs. ARDS group) (Table 2).

Inflammation, apoptosis and oxidation in the plasma and lung tissue

The lung deterioration after the lavage in ARDS group was highlighted by the increase of all observed markers in the plasma and lung vs. Control (Table 3, Fig. 2). In plasma (Table 3), both dexamethasone therapies affected their levels, but only DEX-1.0 significantly reduced TBARS, TNFα and IL-6 (for all $p<0.05$). In the lung tissue (Fig. 2), both DEX therapies reduced the levels of TNFα, IL-8, Casp-3 and TBARS with higher statistical impact for DEX-1.0 vs. ARDS (for TNFα $p<0.001$; for IL-8 and Casp-3 and TBARS $p<0.01$). Only the higher dose DEX-1.0 significantly reduced the level of IL-6 ($p<0.05$) vs. ARDS group (Fig. 2B).

Lung edema and protein content in the BALF

Formation of the lung edema expressed as a wet-dry lung weight ratio (W/D ratio) and protein content in the BALF increased in ARDS group vs. Control (both $p<0.001$). Both DEX therapies comparably decreased W/D ratio (both $p<0.05$) and total protein content (both $p<0.05$) vs. ARDS group (Fig. 2G, 2H).

Discussion

Considering the early phase of ARDS

Table 1. Respiratory parameters: mean airway pressure (MAP), dynamic lung-thorax compliance (Cdyn), airway resistance (Raw), partial pressure of carbon dioxide ($PaCO_2$), oxygen saturation ($SatO_2$), and pH before (basal value, BV) and after induced ARDS (Model) and within 4 h after administration of the therapy (Th) in Control group, ARDS untreated group, and ARDS groups treated with 0.5 mg/kg of dexamethasone (DEX-0.5) or 1.0 mg/kg of dexamethasone (DEX-1.0). Data are presented as means ± SEM.

	BV	Model	30' Th	1h Th	2h Th	3h Th	4h Th
MAP (kPa)							
Control	0.77±0.02	0.74±0.02	0.73±0.02	0.73±0.02	0.71±0.01	0.71±0.01	0.73±0.02
ARDS	0.81±0.03	1.11±0.03†††	1.08±0.03†††	1.07±0.03†††	1.13±0.04†††	1.14±0.04 †††	1.14±0.04†††
DEX-0.5	0.81±0.01	1.06±0.04	0.94±0.04	1.00±0.03	0.91±0.03**	0.94±0.06**	0.97±0.07*
DEX-1.0	0.79±0.05	1.07±0.07	0.97±0.07	0.97±0.07	0.91±0.07**	0.97±0.07*	1.01±0.08
Cdyn (ml/kPa)							
Control	14.5±0.58	15.0±0.38	15.4±0.57	15.3±0.47	14.7±0.68	15.1±0.40	15.3±0.64
ARDS	13.63±0.71	7.13±0.35†††	7.50±0.38†††	6.88±0.40†††	7.25±0.41†††	6.38±0.38†††	6.13±0.40†††
DEX-0.5	13.86±0.26	8.29±0.42	8.43±0.69	8.29±0.87	9.43±0.78	8.71±0.97*	8.43±0.78*
DEX-1.0	13.63±0.75	8.13±0.67	8.75±0.94	8.88±0.97	8.88±0.97	8.75±0.96*	8.75±0.86*
Raw (kPa/l/s)							
Control	4.39±0.22	4.41±0.37	4.84±0.42	4.56±0.35	4.97±0.34	4.61±0.27	4.44±0.31
ARDS	4.84±0.53	11.39±1.92††	12.91±2.09†††	14.57±2.53†††	14.39±2.96†††	14.07±2.84†††	15.02±3.03†††
DEX-0.5	4.72±0.29	7.69±0.65	8.7±0.84	8.99±0.69*	7.66±0.72**	8.58±1.27*	9.07±1.09*
DEX-1.0	4.93±0.32	9.65±1.88	8.94±0.98	8.61±1.20**	8.84±1.30*	8.57±1.28*	8.89±1.30**
$PaCO_2$ (kPa)							
Control	4.17±0.27	4.47±0.27	4.12±0.24	4.18±0.30	4.07±0.32	4.01±0.28	3.76±0.31
ARDS	3.89±0.26	6.79±0.33†††	6.94±0.44†††	6.84±0.35†††	6.67±0.27†††	6.3±0.24†††	6.26±0.33†††
DEX-0.5	3.99±0.08	6.5±0.38	5.97±0.32	6.17±0.37	5.86±0.37	5.94±0.58	5.7±0.50
DEX-1.0	3.87±0.25	6.49±0.27	6.22±0.25	6.02±0.30	5.66±0.32	5.31±0.37	5.1±0.36*
$SatO_2$ (%)							
Control	99.9±0.00	99.9±0.00	99.9±0.00	99.9±0.00	99.9±0.00	99.9±0.00	99.89±0.01
ARDS	99.9±0.00	96.15±0.80	95.85±0.84	90.11±4.19†††	90.85±3.04†††	93.99±1.46†	88.39±5.22†††
DEX-0.5	99.9±0.00	97.39±0.65	97.47±0.72	97.16±0.94*	98.76±0.42**	99.2±0.30	99.49±0.19***
DEX-1.0	99.91±0.01	95.61±3.21	98.86±0.26	98.63±0.41**	99.17±0.26**	99.76±0.05*	99.8±0.05***
pH							
Control	7.47±0.01	7.42±0.02	7.41±0.01	7.37±0.01	7.31±0.03	7.28±0.02	7.26±0.02
ARDS	7.55±0.01	7.23±0.02†††	7.22±0.02†††	7.2±0.03†††	7.15±0.02†††	7.11±0.03†††	7.07±0.02†††
DEX-0.5	7.49±0.03	7.24±0.02	7.24±0.02	7.22±0.03	7.21±0.02*	7.18±0.03*	7.13±0.03*
DEX-1.0	7.5±0.03	7.26±0.02	7.23±0.02	7.22±0.03	7.16±0.04	7.16±0.03	7.15±0.03*

characterized by neutrophil-mediated inflammation, lung cell injury and apoptosis with subsequent influx of protein-rich fluid into the alveoli and edema formation, therapy with glucocorticoids (GC) may be of benefit, due to their potent anti-inflammatory and anti-edematous properties (Coutinho and Chapman 2011). Positive effects of GC therapy have been previously shown in various models of lung injury (Wang *et al.* 2005, Beck *et al.* 2009, Newton *et al.* 2010) and their role was found in favor of treatment of ARDS (Peter *et al.* 2008). There are evidences for the beneficial role of dexamethasone (DEX) in ARDS condition (Villar *et al.* 2016, Qin and Qiu 2019), particularly for low dose of DEX (Lee *et al.* 2005, Xinmin *et al.* 2006, Wang *et al.* 2008). Controversial results found in the previous studies on the use of GC could arise from the different study designs and etiology of ARDS as well as from different dosage and timing of administration (Ruan *et al.* 2014).

Table 2. Total and differential leukocyte count in the arterial blood before (basal value, BV) and in the 4 h of the therapy (Th); and in the bronchoalveolar lavage fluid (BALF) in Control group, ARDS untreated group, and ARDS groups treated with 0.5 mg/kg of dexamethasone (DEX-0.5) or 1.0 mg/kg of dexamethasone (DEX-1.0). Data are presented as means ± SEM.

ARTERIAL BLOOD					
		Control	**ARDS**	**DEX-0.5**	**DEX-1.0**
Total count of leukocytes (x10^6/ml)					
	BV	1.99±0.37	3.26±1.05	2.57±0.57	4.24±0.57
	4h Th	3.11±0.81	1.29±0.25†	1.98±0.42	1.84±0.53
Differential count (%)					
Neutrophils	BV	2.54±0.81	1.84±0.59	2.04±0.45	3.53±0.90
	4h Th	51.79±6.49	21.56±3.09††	50.64±5.90*	44.07±4.94*
Lymphocytes	BV	92.91±1.86	96.27±0.87	93.5±2.08	93.48±1.16
	4h Th	42.1±5.41	79.87±2.34††	47.59±5.78*	53.74±4.78*
Monocytes	BV	1.3±0.25	0.93±0.13	1.18±0.19	0.98±0.12
	4h Th	1.2±0.19	0.77±0.16†	1.07±0.20	1.49±0.22*
Eosinophils	BV	0.84±0.27	0.96±0.39	1.38±0.50	0.96±0.19
	4h Th	0.36±0.12	0.39±0.17	0.7±0.18	0.7±0.23
BALF					
		C	**ARDS**	**DEX-0.5**	**DEX-1.0**
Total count (x10^3/ml)		2.29±0.29	63.33±9.67†††	8.14±2.40*	3.63±0.80**
Monocytes (%)		96.51±1.14	20.43±4.32†††	33.6±10.07	56.1±6.35*
Neutrophils (%)		2.74±0.87	76.67±4.57†††	63.5±9.67	39.87±5.72*
Eosinophils (%)		0.74±0.28	2.91±0.72†	2.74±0.56	2.77±0.56

Table 3. Oxidative and inflammatory markers in the plasma: 3-nitrotyrosine (3NT), thiobarbituric acid-reactive substances (TBARS, and cytokines TNFα, IL-6 and IL-8 in Control group, ARDS untreated group, and ARDS groups treated with 0.5 mg/kg of dexamethasone (DEX-0.5) or 1.0 mg/kg of dexamethasone (DEX-1.0). Data are presented as means ± SEM.

	3NT	**TBARS**	**TNFα**	**IL-6**	**IL-8**
Control	18.09±0.3	6.43±0.8	14.5±6.8	9.67±2.1	424.1±75.1
ARDS	21.88±1.7†	9.97±1.2†	106.8±18.6††	44.97±8.2††	1050.0±116.7††
DEX-0.5	18.3±0.5*	7.22±0.3	81.53±22.3	22.41±4.6	550.6±133.8
DEX-1.0	18.9±0.9	6.69±0.6*	64.45±24.9*	13.56±4.3*	634.1±202.5

The effective dosage of DEX and action in early stage of developing ARDS is still unresolved and definite conclusion cannot be made. Thus, our study has focused on efficiency of two different low bolus doses of dexamethasone (0.5 and 1.0 mg/kg) administered intravenously directly after developing ARDS condition, where anti-inflammatory and anti-edematous activity of DEX was expected to improve finally the lung function in experimental ARDS.

ARDS is associated with a diffuse alveolar epithelial and endothelial damage leading to an influx of fluid and activated inflammatory cells into the alveoli what could negatively affect a surfactant function and thus the respiration (Ranieri *et al.* 2012). In our study, repeated lung lavage with a saline led to deterioration of the lung function parameters (P/F ratio, OI, VEI, AaG, MAP, C_{dyn}, Raw, $PaCO_2$) within minutes after the insult what is consistent with the findings of other authors (Kamiyama *et al.* 2015, Ricci *et al.* 2017). Administration of DEX in both doses improved the lung function parameters. However, rapid improvement in P/F ratio and oxygenation index was observed within the first 30 min after administration of the higher dose of DEX-1.0, which persisted till the end of experiment. Significant effect of

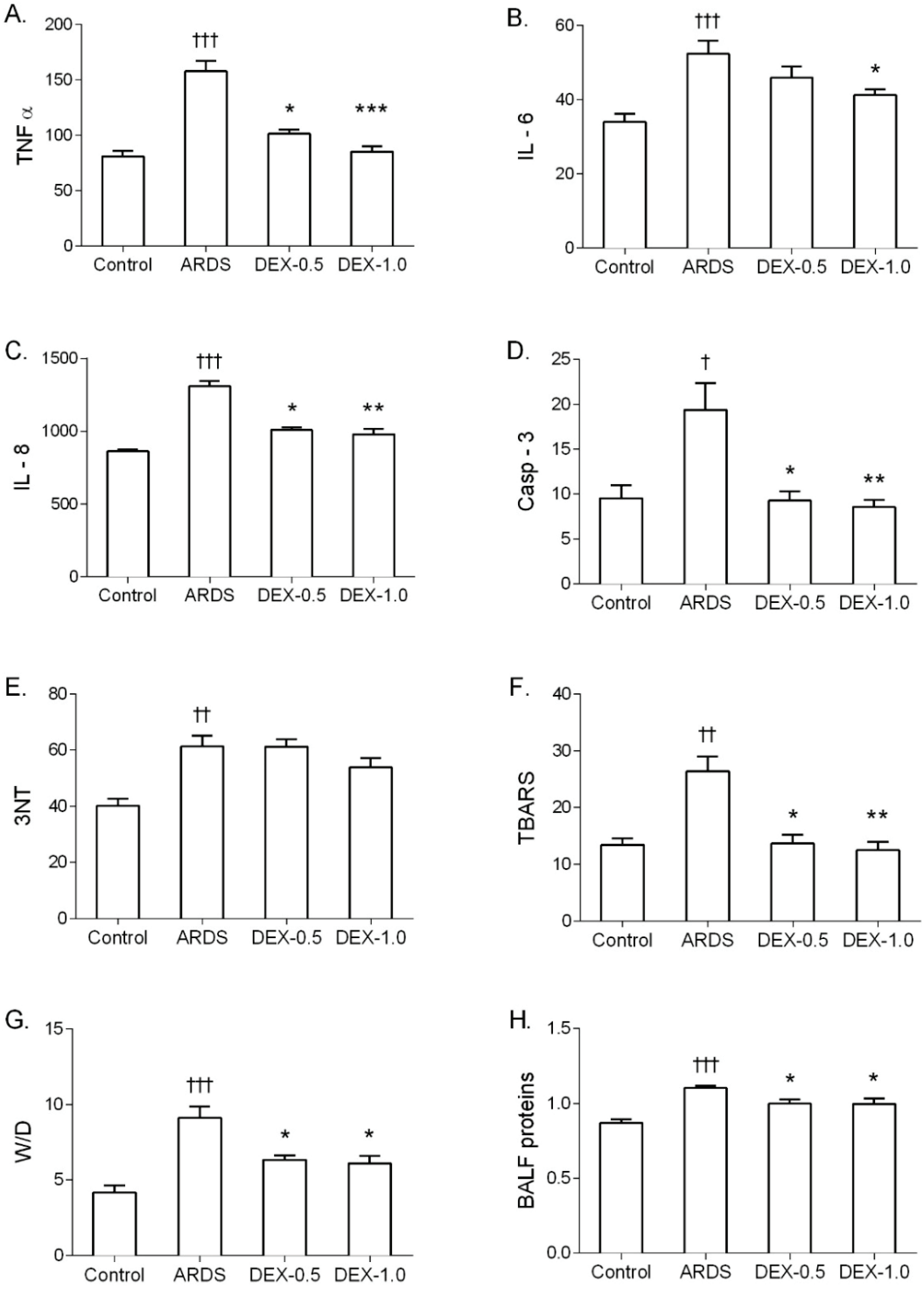

Fig. 2. Levels of cytokines (**A**) TNFα, (**B**) IL-6 and (**C**) IL-8; (**D**) marker of apoptotic process caspase-3 (Casp-3); (**E**) protein oxidation (concentration of 3-nitrotyrosine, 3NT) and (**F**) lipid oxidation (thiobarbituric acid-reactive substances, TBARS); (**G**) lung edema formation expressed as wet-dry (W/D) lung weight ratio; (**H**) protein content in bronchoalveolar lavage fluid (BALF) in lung tissue homogenate in Control group, ARDS untreated group, and ARDS groups treated with 0.5 mg/kg of dexamethasone (DEX-0.5) or 1.0 mg/kg of dexamethasone (DEX-1.0). Data are presented as means ± SEM. Statistical comparisons: for ARDS vs. Control † $p<0.05$, †† $p<0.01$, ††† $p<0.001$; and for DEX-0.5 & DEX-1.0 vs. ARDS * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

DEX-1.0 was underlined in P/F ratio and alveolar-arterial gradient (AaG) at 3 h of the therapy compared to lower dose. In addition, only the higher dose of DEX improved VEI compared to ARDS group. Previous studies demonstrated positive effects of GC in experimental (Mokra *et al.* 2007, Kosutova *et al.* 2016) and clinical studies (Meduri *et al.* 2007, Tang *et al.* 2009). GC could enhance the lung function improving oxygenation and

ventilatory parameters leading to reduction of period of oxygen dependency and duration of ICU and hospital stay.

The ARDS is associated with influx of leukocytes into the alveolar spaces. We observed a significant increase of total leukocytes count, neutrophils and eosinophils in the BALF in ARDS group compared to controls what is in agreement with other studies (Noda *et al.* 2003, Waragai *et al.* 2007, Abdelmageed *et al.* 2016). Administration of the higher dose of DEX significantly affected the leukocytes in the BALF by decreasing their total count and neutrophils as we observed previously (Mokra *et al.* 2007, Kosutova *et al.* 2016).

Subsequent neutrophils activation in the lung leads to inflammation which plays a key role in a progression of ARDS. In our experiments, increased levels of pro-inflammatory cytokines (TNFα, IL-6 and IL-8) in the plasma and lung tissue homogenates of ARDS animals were detected, similarly to results of other authors (Kalk *et al.* 2008, Kamiyama *et al.* 2015). The inhibitory effects of GC on the inflammation are partially mediated through an inhibition of the synthesis of NF-κB dependent pro-inflammatory cytokines (Coutinho and Chapman 2011). In this study, DEX administration decreased levels of pro-inflammatory cytokines in the lung and plasma compared to the ARDS group, while the higher dose DEX-1.0 had slightly stronger impact on these changes. The effectiveness of dexamethasone in reducing levels of cytokines was observed also by other authors (Xinmin *et al.* 2006, Qin and Qiu 2019).

Overzealous activation of neutrophils could lead to the tissue damage through protein and lipid oxidation (Matthay and Zemans 2011). In our experiments, protein nitrosylation (expressed by 3NT) and lipid peroxidation (expressed by TBARS) significantly increased in the lung and plasma after the lavage-induced lung injury. Neutrophil-induced damage of proteins and lipids through their oxidation was previously published (Lang *et al.* 2002). Generated ROS have indirect pro-inflammatory effects and together with inflammation participate in activation of caspase-3, which causes the direct epithelial injury *via* apoptotic pathways (Galani *et al.* 2010). An increased level of caspase-3 in the ARDS animals in our study indicates an activation of pro-apoptotic processes early after the triggering injury. DEX of both dosages decreased the markers of oxidative damage in the plasma, while TBARS and caspase-3 significantly decreased also in the lung tissue. Similarly to our results, dexamethasone alleviated inflammation and suppressed Fas ligand and thus extrinsic apoptotic pathway in other experimental ARDS model (Beck *et al.* 2009) and inhibited an activation of caspase-3 and -7 in the lung epithelial cells (Wen *et al.* 1997).

The inflammatory mediators and bioactive substances including ROS damage the endothelial and epithelial cells and thereby increase the permeability across the alveolar-capillary membrane, resulting in the pulmonary edema formation (Matthay and Zemans 2011). In this study, Degree of the lung edema formation calculated as a ratio of wet and dry lung weight (W/D) and estimated also using total protein content in BALF significantly increased in the ARDS group, similarly to other authors (Li *et al.* 2016). Both dosages of DEX significantly decreased the lung edema formation and the protein content in the BALF compared to untreated animals.

Nevertheless, our findings are in conflict with another very similar study, where systemic dexamethasone did not have any effect on clinic parameters and lung injury (Engel *et al.* 2015). The discrepancy may be related to different experimental animals used in these studies and varied lung lavage and treatment protocol. On the other hand, other several studies showed positive effect of low-dose steroids on early phase ARDS (Annane *et al.* 2006, Meduri *et al.* 2007, Meduri *et al.* 2008, Wang *et al.* 2008, Tang *et al.* 2009). Taken together, dexamethasone can be of benefit in the therapy of ARDS. Both low doses of DEX could prevent migration of polymorphonuclear leukocytes into the lung and modulate their activation what could inhibit the local inflammation and alleviate respiratory failure.

In this study, the higher dose of DEX (1.0 mg/kg) showed more obvious improvement in the parameters of oxygenation and VEI with faster onset of the action than the lower dose of DEX (0.5 mg/kg) in the early phase ARDS, however, this effect was only temporary. Therefore, we can speculate that the use of lower dose of DEX (0.5 mg/kg) can be considered for more reasonable as it provides nearly comparable effects on the lung function, inflammation, oxidation and lung edema formation to higher dose of DEX (1 mg/kg), but in expected lower risk of occurrence of the side effects, whereas the development of side effects of GC is time- and dose-dependent. Even though the early and low-dose dexamethasone during early phase ARDS had positive effect consistent with a meta-analysis of recent randomized controlled trials (Yang *et al.* 2017), further research in this field is necessary.

Declaration of Interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Conflict of Interest

There is no conflict of interest.

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