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Pulmonary Haemodynamics in Acute Experimental Lung Vascular Injury

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Summary

Acute lung injury was induced by intravenous injection of 20 μl of a mixture of equivalent volumes of capronic acid, caprilic acid and olive oil in intact anaesthetized rats and in isolated perfused rat lung preparations. Lung injury in intact rats resulted in an increase in lung weight related to body weight and in a decrease in the lung dry/wet weight ratio. Lung compliance, measured in a body plethysmograph, was decreased. Pa_{O_2} decreased and Pa_{CO_2} increased in 10 and 20 min, respectively, after the beginning of the experiment. Mean blood pressure in pulmonary artery increased immediately after the injection. Isolated rat lungs were perfused at constant flow with physiological saline solution containing bovine albumin and meclofenamate. The injection of a mixture of capronic acid, caprilic acid and olive oil increased the baseline perfusion pressure and led to a release of endothelial cells into the perfusate. The perfusion flow-pressure relationship was shifted upwards. Both the extrapolated pressure axis, intercept and slope of the plot were significantly elevated. The described experimental lung injury is a suitable model for studies on the effects of vascular wall damage and transvascular fluid leak in pulmonary vasculature.

Key words
Lung injury – Lung oedema – Pulmonary haemodynamics – Rat

Introduction

Lung vascular injury causes morphological transformation of the pulmonary vascular bed which is the main pathogenetic mechanism of pulmonary hypertension (Reid 1986, Herget and Ježek 1989, Belik *et al.* 1994, Herget and Hampl 1995). Repeated experimental lung injury in rats causes chronic pulmonary hypertension (Herget *et al.* 1981, Hill *et al.* 1984). Lung endothelial damage and consequent transvascular fluid leak are considered to be important mechanisms of the remodelling of peripheral pulmonary arteries (Herget and Ježek 1989). The aim of the present study was to characterize an experimental model of acute lung injury which would be a suitable approach for further studies on the role of endothelial damage and increase of vascular wall permeability in remodelling of the pulmonary vasculature and in the development of pulmonary hypertension.

Methods

Lung injury

Lung injury was induced by intravenous injection of 20 ml of a mixture of equivalent volumes of capronic acid, caprilic acid and olive oil (Bost *et al.* 1969). The mixture was injected into the jugular vein or pulmonary artery in anaesthetized (thiopental 50 mg kg/b.w i.p) intact rats or into the inflow cannula of a preparation of isolated perfused rat lungs.

Measurement of lung function in intact animals

Seven anaesthetized male Wistar rats (body weight 290±4 g) were intubated and the jugular vein was exposed. The animals were placed in a body plethysmograph (Paleček 1969) and control values of tidal volume (V_T), rate of breathing (f) and dynamic lung compliance ($C_L = V_T/P_{oes}$, where P_{oes} is the difference of oesophageal pressure in expirium and inspirium) were measured. A bolus of 20 μl of the mixture of capronic acid, caprilic acid and olive oil was

rapidly injected into the jugular vein. Measurements of lung function were repeated at 30 s, and 5, 10, 20 and 30 min after the injection. The rats were then heparinized (1000 IU), 30 mg of thiopental was given into the jugular vein and both the vena cava and abdominal aorta were cut with scissors to exsanguinate and minimize the effect of blood volume on lung weight (Richter 1952). After thoracotomy, the right lungs were isolated, weighed and dried to constant weight (approx. 2 h) in a microwave oven. Ten right lungs of untreated rats were obtained from another experiment and served as controls.

Pulmonary artery pressure

Six male Wistar rats (body weight 265±9 g) were used after similar anaesthesia as in the ventilatory experiments. The pulmonary artery was catheterized *via* the right jugular vein (Herget and Paleček 1972). A cannula was inserted into the left femoral artery and the animals were heparinized. Blood samples of 100 μl for Pa_{O_2} and Pa_{CO_2} measurement were obtained from the femoral artery. The mixture of capronic acid, caprilic acid and olive oil was injected into the catheter in the pulmonary artery and flushed in with 0.1 ml of saline. The values of pulmonary artery pressure, Pa_{O_2} and Pa_{CO_2} were measured at similar intervals as those described for ventilatory measurement. At the end of the experiment the animals were sacrificed by thiopental overdose.

Experiments on isolated lungs

Five male Wistar rats (body weight 305±11 g) were used. The preparation of isolated perfused lungs was made as described previously (Hampl and Herget 1990). Lungs were ventilated with air + 5 % CO_2 at 65 breaths per min. They were then perfused with a physiological saline solution containing 4 g/100 ml of bovine albumin (Sigma, fraction V) and 0.016 mM sodium meclofenamate (Herget and McMurtry 1985). The rate of perfusion was 0.06 ml/min/g of body weight. After 20 min stabilization, the perfusion pump was stopped for about 30 s and then the perfusion flow was increased in 5 steps (30–90 s each) up to 37 ml/min, to measure the perfusion flow-perfusion pressure relationship. The perfusate from the venous reservoir was sampled for counting endothelial cell carcasses (ECC). Then 20 μl of the mixture of capronic acid, caprilic acid and olive oil were injected into the inflow cannula. After 15 min of perfusion, the measurement of perfusion flow-pressure relationship was repeated. The perfusate was sampled again at 20 min after the lung injury for estimation of the number of ECC in the perfusate. The count of circulating endothelial cell carcasses was made according to the method of Hladovec and Rossman (1973).

Statistics

Data are presented as means ± S.E.M. They were analysed by ANOVA and paired and unpaired t-tests. The individual flow-pressure curves were analysed by linear regression and were found to be linear ($r>0.925$). Therefore, the slope and intercept with the pressure axis were measured. The difference between flow-pressure relationships before and after lung injury was analysed by ANOVA and Dunnet's multiple comparison test. The statistical analyses were made with statistical software StatView 4.53, Abacus Concepts. Values $p<0.05$ were considered to be significant.

Table 1. Values of tidal volume (V_T), rate of breathing (f), lung compliance (C_L), Pa_{O_2} and Pa_{CO_2} after acute lung injury

	Control	1 min	10 min	20 min	30 min
V_T (ml)	1.1±0.1	1.2±0.1	1.2±0.1	1.1±0.1	1.1±0.1
f (c/min)	108±12	126±9	137±9	152±16*	146±12*
C_L (ml/cm H_2O)	0.23±0.02	0.17±0.01*	0.17±0.01*	0.16±0.02	0.16±0.02
Pa_{O_2} (mm Hg)	83.3±5.6	–	83.3±5.6	72.5±4.8*	61.2±3.0*
Pa_{CO_2} (mm Hg)	41.8±1.5	–	47.3±0.5*	51.1±1.2*	53.7±1.6*

*The values before lung injury (Control) and 1, 10, 20 and 30 min after the injection of the mixture of capronic acid, caprilic acid and olive oil. * $p<0.05$ compared with the control value*

Results

The right lung weight related to body weight in rats with lung injury was significantly greater (0.55±0.07 %) than in control rats (0.34±0.04 %, $p<0.002$). The dry weight/wet weight ratio was significantly lower in the experimental group (18.3±1.2 % in rats with lung injury and 21.9±0.6 % in control rats, $p<0.05$). There were no changes in tidal volume. The rate of breathing was significantly

increased 20 and 30 min after the injection of capronic acid, caprilic acid and olive oil. Lung compliance decreased one and 10 min after the onset of lung injury. The partial pressure of oxygen significantly decreased at 20 and 30 min; the Pa_{CO_2} progressively rose from the 10th min after of the lung injury (Table 1). The mean pulmonary artery pressure increased at 30 s and remained significantly higher up to 20 min after the beginning of the injury (Fig. 1).

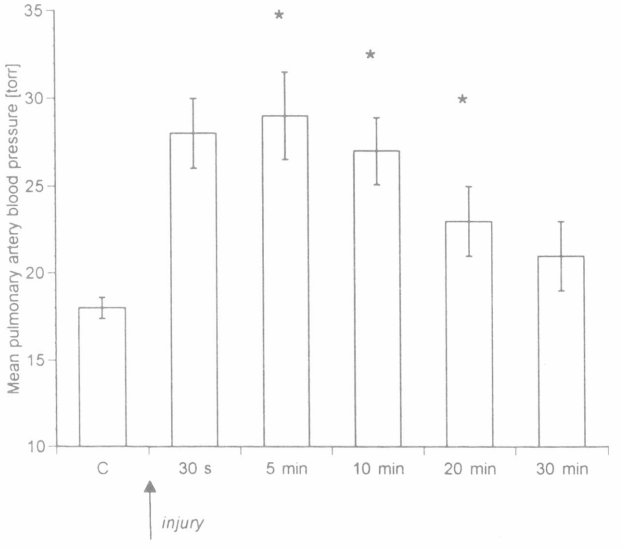

Fig. 1. *Changes of the pulmonary mean blood pressure after lung injury. * $p<0.05$.*

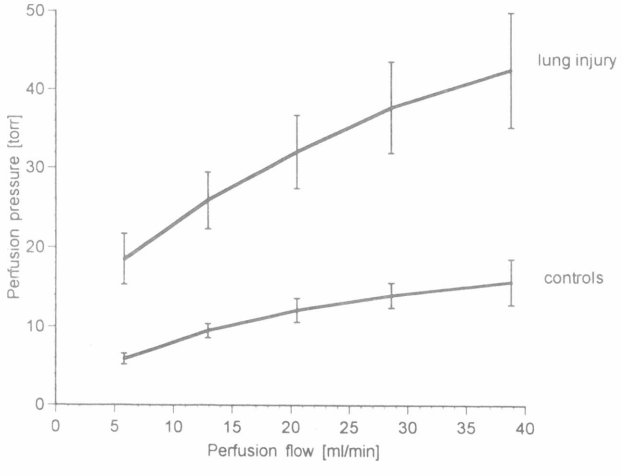

Fig. 2. *Perfusion flow-perfusion pressure relationship in isolated perfused lungs with acute lung injury. ANOVA and multiple comparisons test indicates the significant difference between the control flow-pressure plots and plots after lung injury.*

The number of endothelial cell carcasses in the outflow blood in isolated lungs was significantly higher ($490 \pm 20 \times 10^4$ ECC/ml) 20 min after lung injury than before the injection of capronic acid, caprilic acid and olive oil ($210 \pm 20 \times 10^4$ ECC/ml, $p<0.05$, paired t-test). The perfusion pressure-flow lines measured 15 min after beginning of the lung injury were significantly shifted upwards compared to the control curve measured before the lung injury (Fig. 2). Both slopes and pressure axis intercepts were significantly increased after lung injury. Before lung injury, the slope of curve was $+0.308 \pm 0.037$ mm Hg.$(ml.min)^{-1}$ and the intercept was $+4.944 \pm 0.91$ mm Hg. After lung injury they were $+0.771 \pm 0.06$ mm Hg.$(ml.min)^{-1}$ and $+15.25 \pm 3.2$ mm Hg, respectively.

Discussion

The injection of capronic acid, caprilic acid and olive oil resulted in serious injury of the lungs accompanied by a transvascular fluid leak. The dry to wet weight ratio of the injured lungs was decreased and the relative lung weight was higher than in control rats. The decrease of dynamic lung compliance might be explained by interstitial lung oedema and hyperaemia (Hughes *et al.* 1958). The values of lung compliance at the end of experiment did not differ significantly from the control values. The likely explanation, however, is the large variability of the values. Interstitial lung oedema probably also explains the increased rate of breathing (Paintal 1973). Arterial hypoxaemia and hypercapnia may be attributable to venous admixture in the unevenly ventilated and perfused lungs. In a very similar model induced by oleic acid in dogs, Leeman *et al.* (1989) have found that the venous admixture increased from 5 to 54 % after lung injury. Diffusion impairment may be considered as an additional factor. Minute ventilation did not change significantly during the experiment. Pulmonary artery pressure rose after the injection of capronic acid, caprilic acid and olive oil. The decrease of pulmonary artery pressure at the end of the experiment may be attributed to the possible decrease of lung blood flow. The cardiac output, however, was not measured. Because of the very small volume injected, it is unlikely that this can be explained by vascular embolization. The increase in pulmonary artery pressure was also found in dogs injected with oleic acid (Leeman *et al.* 1989). A direct toxic effect on the endothelial cells is a more probable explanation. A similar effect was seen in the saline-perfused isolated lungs which had meclofenamate in the perfusate. Therefore, blood-born mediators or products of the cyclooxygenase pathway are probably not involved. The increase in endothelial desquamation after lung injury may be the result of pulmonary vasoconstriction and/or may reflect an unknown toxic effect on endothelial cells.

Both the extrapolated pressure (zero flow) on the ordinate and the slope of the perfusion pressure flow lines were significantly increased after lung injury. The pressure at zero flow represents the mean closing pressure of pulmonary blood vessels. This is due to a balance of forces on the collapsible (middle) part of

the pulmonary vasculature (Permutt and Riley 1963). The increase of the intercept after lung injury resulted probably not only from an increase in active vascular tension (vasoconstriction) but also from an increase of perivascular pressure due to perivascular lung oedema (West *et al.* 1965). The increase in the slope of the perfusion flow-pressure plot may result from a decrease in pulmonary vascular compliance due to pulmonary oedema and/or to an increase in resistance to changes of blood flow in the noncollapsible (upstream) part of the pulmonary vasculature. Vasoconstriction of extraalveolar vessels or encroachment of vascular wall into the lumen may explain the increase of vascular resistance. The double occlusion technique, which enables to measure the distribution of vascular resistances and compliances along the pulmonary vascular bed (Hakim *et al.* 1982, Herget and Hampl 1995), might provide more specific information about the rheological changes induced by this type of lung injury.

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