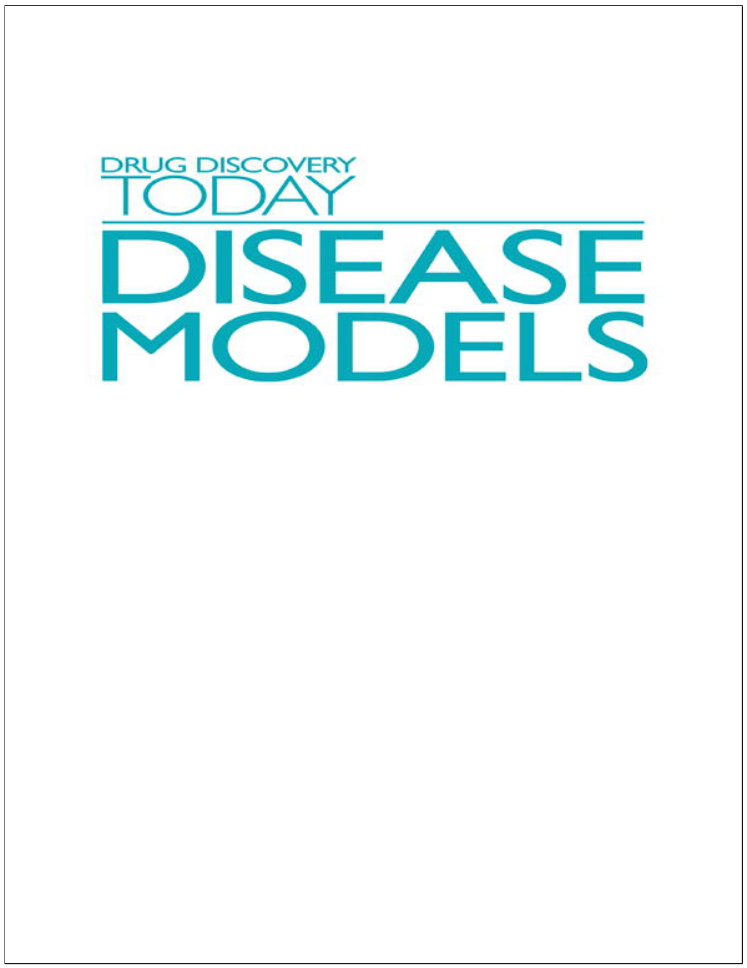

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DRUG DISCOVERY TODAY DISEASE MODELS

Drug Discovery Today: Disease Models
Vol. 7, No. 3–4 2010

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Pulmonary vascular disease

Isolated perfused murine lung

A well characterized preparation for studying lung vascular function

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Preparations consisting of an isolated perfused lung from small laboratory animals is useful tool to study local regulatory mechanisms in health and disease. Isolated lung can be perfused with blood or with salt solution including albumin for several hours without functional impairment. The basic advantage of this preparation is the good opportunity to control individual hemodynamic and ventilation parameters in well defined experimental conditions.

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Introduction

Isolated perfused lungs are powerful tool for investigating pulmonary function, structure and local regulatory mechanisms. In comparison to '*in vivo*' studies, the preparation is studied in isolation from other organs and from their effects. Both basic functions, lung ventilation and lung perfusion, can be controlled and measured with reasonable accuracy in preparations obtained from small and inexpensive laboratory animals (rats and mice). Inputs to the system, for example, composition of the perfusate and inspired gas, can be altered according to the objective of the experiment. The transfer of any substrates applied in the extracellular solution occurs via the capillary vasculature in a physiological process. Of course isolated perfused lung possess all of the disadvantages of studies performed on laboratory animals, such as the limits in transfer of findings in human medicine.

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The absence of central integrative mechanisms (both neural and humoral) restricts the obtained view of the organ at the local level. In contrast with other '*in vitro*' methods, the cells in the isolated perfused lung are maintained in their normal anatomical and functional associations. Transcellular transport and diffusion mechanisms occur as they would *in vivo*. Compared with tissue slices or even less organized lung tissue samples, the isolated perfused lung still retains all the basic lung functions, including the exchange of respiratory gases between the atmosphere and the lung perfusate. Preservation of this ability is a key aspect of the viability of the preparation. Last but not least, the currently most widely used preparation of the isolated perfused lung is an uncomplicated technique and does not require special skills or laboratory equipment (Table 1).

General principles of the 'in vitro' *system*

Since the earliest experiments on isolated perfused lungs carried out as early as at the beginning of the last century [1], the basic principle of the technique has not changed much. Typically, the ventilated lungs are perfused through a cannula inserted in the pulmonary artery. The perfusate is collected in a venous reservoir by a cannula inserted in the

1740-6757/$ DOI: 10.1016/j.ddmod.2011.03.008

Table 1. Advantages and disadvantages of preparation of isolated lungs

Advantages	
Controlled	– perfusion flow or pressure
	– composition of perfusate
	– mechanics of breathing
	– composition of ventilatory gases
	– temperature
Perfusion flow	– pressure relation
Occlusion techniques	
Low cost	
Simplicity and reproducibility	
Disadvantages	
Isolated organ	– no neural control
	– local humoral regulation only
	– absence of heart pump
	– effects of mechanical pumping
	– limited defense mechanisms
Limited time of viability	
Limited energy stores in perfusate	

left heart. Perfusion is typically performed in a recirculating manner (Fig. 1). Lungs (or separated lung lobes) of most species of experimental animals have been used in the past. Human lungs obtained after resection because of bronchial carcinoma were also successfully tested [2]. In some experiments, isolated perfused lungs were used as an 'oxygenator' for other organs [3].

Lungs can be studied exteriorly from the thorax and placed in a warm and humid chamber. Less often they can be left '*in situ*'. We have personal experience with both, and we did not find any difference, apart from that the fact that the first alternative is technically more simple.

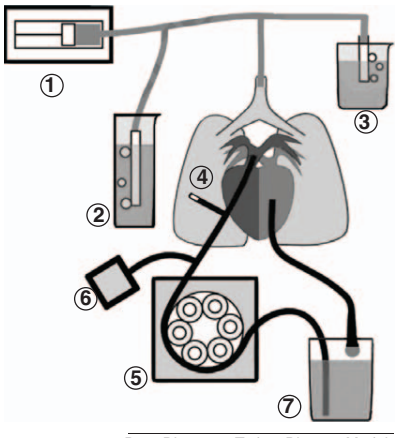

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Figure 1. Schema of isolated perfused lung set-up. 1: ventilation pump, 2: inspiration positive pressure valve, 3: end expiratory positive pressure valve, 4: air bubbles trap, 5: peristaltic pump, 6: perfusion pressure manometer, 7: venous reservoir.

Procedure for the preparation of rat isolated perfused lungs

An anesthetized rat is placed in the supine position and secured to the table. Then a cannula is introduced into the trachea and, after medial thoracolaparotomy, it is connected to the ventilator (no positive expiratory pressure is applied at this time). The thorax is supported in a wide open position. A ligature is positioned together under the aorta and the conus of the pulmonary artery as near to the right ventricle as possible and around the heart apex. Heparin in physiologic saline solution (250 I.U.) is injected into the right heart ventricle. Right and left heart ventricles are incised and arterial (inflow in pulmonary artery) and venous (outflow from the left ventricle) cannulae are introduced (Fig. 2a). The entire heart–lung block is then excised from the animal's body. The tracheal cannula connected to the ventilator and the preparation is suspended in a humid chamber (Fig. 2b). Positive end expiratory pressure is restored while the perfusion flow is slowly increased up to the desired value. It is important to start the heating of the preparation at this point. Effluent perfusate is drained from the left ventricular cannula into a reservoir; the outflow pressure is set to −2 mmHg. The first 20 ml of artificial perfusate through the lungs is discarded, leaving a recirculating volume of 30 ml. Then, the lungs are allowed 20 min for stabilization. The preparation should be carefully monitored during the stabilization period with special attention focused on a possible blockade in the outflow tract. At the beginning of perfusion, the contracture of the left heart ventricle may obstruct outflow cannula, which results in an increase in perfusion pressure and the formation of lung edema. Another problem which may appear is the formation of air bubbles in perfusate. Air bubbles can be easily caught by a bubble trap (T piece positioned between the peristaltic pump and arterial cannula).

Regulation of lung perfusion in isolated lungs

Collected blood is the most natural fluid for perfusion. For rat and mouse preparations, a sufficient volume of blood for the circuit can be obtained by cardiac puncture from anaesthetized blood donors. The blood donors can be used repeatedly after an interval of a few weeks. An alternative perfusion fluid is a physiologic salt solution combined with plasma expanders (bovine serum albumin or Ficoll). An artificial medium widely used and recommended for rat and mouse lung perfusion is Krebs-Hensley bicarbonate buffer containing bovine serum albumin (Table 2) [4].

The advantage of the blood perfused preparation is that it preserves the vasoconstriction in response to acute hypoxia to such an extent that it is comparable with that observed in '*in vivo*' conditions. In saline perfused lungs, the normal reactivity to hypoxia requires prestimulation with excitatory substances such as plasma, angiogenesis II, PGF2α, ACL or glucocorticoids [5,6]. The same is true for isolated pulmonary blood vessels studied in the salt solution. The disadvantages

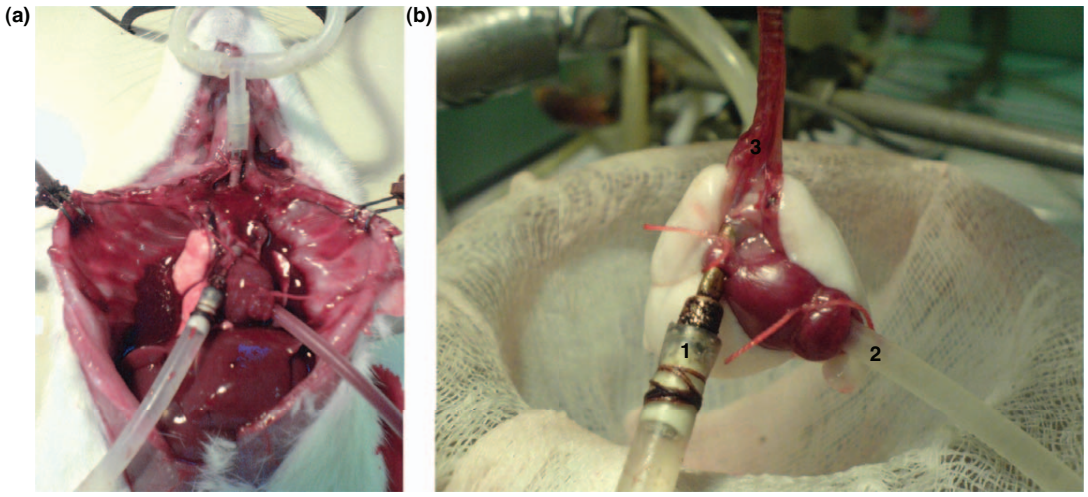

Figure 2. Procedure for the preparation of rat isolated perfused lungs. (a) Lungs and heart in chest cavity. (b) Inflow (1), outflow (2) and tracheal (3) cannula positioned in preparation on top of heated and humid chamber.

of using homologous blood for perfusion are undefined blood composition and uncertainty concerning the state of the blood donor. Furthermore, due to the limited energy sources, blood perfusate is not suitable for long lasting experiments [7].

The perfusate is circulated by a pump and various models of suitable peristaltic (roller) pumps are available. The advantage of peristaltic pump is absence of valves and therefore the only part of the pump in contact with perfusate is interior of tube. It makes the system easy to clean. In blood perfused preparations, the combination of fast roller speed and thin tubing decreases pulsations in the circuit but increases the risk of mechanical damage to formed blood elements. Mechanical pumping may result in platelets damage, erythrocyte hemolysis and alteration of polymorphonucleocytes [8]. Estimation of perfusion flow from roller speed is not always accurate, and we recommend a simultaneous direct measurement with the flowmeter included in the circuit. The perfusate is most often used in a recirculating fashion between the lungs and a heated venous reservoir. This technique, despite some of its disadvantages (accumulation of metabolites, exhaustion of substrates and possible accumulation of damage to the perfusion medium by the circulatory pump) is used most often because a large amount of perfusate is needed in non-recirculating perfusions. In addition, in one pass perfusion the fluid constantly changes, therefore the equilibrium between the perfusate and lung tissue cannot be readily achieved [9].

Table 2. Composition of salt solution containing albumin for isolated perfused rat lung (in mM)

NaCl	119
KCl	4.7
$MgSO_4$	1.17
$NaHCO_3$	22.6
KH_2PO_4	1.18
$CaCl_2$	3.2
Glucose	5.5
Bovine serum albumin (Sigma, fraction V) or Ficoll[a] 4 g/100 ml of solution	

[a] When Ficoll is used, the solution contains 19 mM $NaHCO_3$ and 1.6 mM $CaCl_2$.

The perfusion can be controlled by perfusion flow (constant flow preparation) or by perfusion pressure (constant pressure preparation). The first arrangement is more simple: the flow of perfusate is directly led to a cannula positioned in the pulmonary artery, and because the flow is constant, the increase in perfusion pressure represents the increase in the resistance to flow. In the second alternative, the perfusion pressure is set to a constant value and an electronic feedback mechanism decreases perfusion flow if the resistance to flow increases (Fig. 3). The pressure controlled peristaltic pumps are commercially available. We are of the opinion that the 'constant pressure' is a better model of physiologic control of lung perfusion. In typical lung regulation, vasoconstriction results in the restriction of downstream perfusion flow and thus regulates the distribution of pulmonary blood flow. There is lower tendency to develop the lung edema after vasoconstriction in 'constant pressure' conditions.

In constant flow preparations, we recommend to begin with low flows of perfusate (about one fourth of the final value). Then, over a period of 10 min, flow is gradually increased to the final value. In rat and mouse isolated lungs, we use a constant perfusion flow of 4 ml/min/100 g body weight of blood or physiologic salt solution perfusate with albumin. Flows ranging from 6 to 15 ml/min for adult rats have been reported; they are dependent upon the apparatus being used in the experiment [6,7,10–18]. Comparable flow

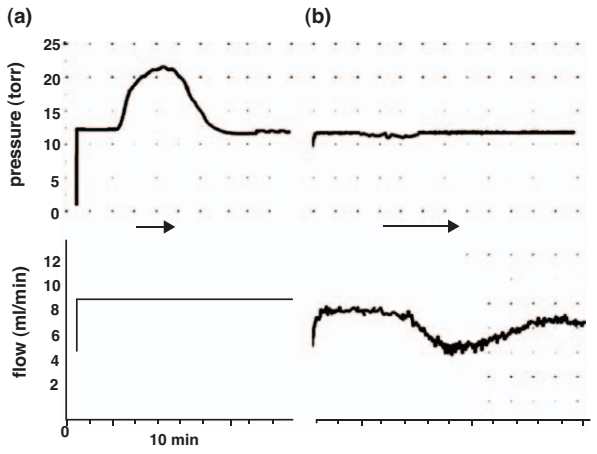

Figure 3. Hypoxic pulmonary vasoconstriction in isolated blood perfused lungs of adult rat studied in constant flow (10 ml/min) (A) and constant pressure (12 Torr) mode (B). (→) ventilation of lungs with 3% O_2 + 5% CO_2 balanced with N_2.

related to body weight is used in mouse preparations [19]. The outflow pressure is set to −2 mmHg.

Utilizing this system of isolated perfused lungs allows more detailed analysis of lung hemodynamic. The ability to measure, for example, the relationship between perfusion flow and perfusion pressure [11,20] or analysis of distribution of pulmonary vascular resistances by the double occlusion technique [11,21,22]. These methods helped to identify the site of action of physiologic vasoconstrictors (e.g. acute and chronic hypoxia, serotonin, etc.) and to understand the regulatory role of interactions between the individual parts of pulmonary vascular bed.

We discard preparations which, after 20 min of stabilization, have very high basal perfusion pressure (more than 12 Torr with saline perfusate, or more than 17 Torr perfused with blood).

Regulation of ventilation in isolated lungs

As previously mentioned the lungs are ventilated via a cannula inserted into the trachea. Ventilation can be accomplished by the periodic inflation of the lung by positive pressure or by the lung expansions induced by negative pressure pulses in the container in which the lung is suspended. The air which surrounds the lung and the inspired gas must be preheated to body temperature and humified. Preheating of the gas prevents water condensation in the ventilation tubing. There is no substantial difference in pulmonary vascular resistance between positive and negative pressure breathing [17]. Most laboratories have adopted positive pressure ventilation because of technical complications incidental to the negative pressure system. Lungs must be placed in a sealed chamber, which makes all manipulations and sampling inconvenient.

In the positive pressure ventilated lung, the peak inspiratory pressure is set to +10 cm H_2O by a pressure water valve, the end expiratory pressure is set to +2 cm H_2O. The usual ventilation frequency is 50 c/min. During positive pressure ventilation, the changes in the mechanical properties of the lung can be monitored by the Konzett method [23]. The composition of the most frequently used gas for the ventilation of rat and mouse isolated perfused lungs is 21% O_2 + 5% CO_2 + 74% N_2. No difference in lung viability was found between ventilation with gas containing 21% O_2 and 95% O_2 [24]. Withdrawal of CO_2 leads to alkalosis and lung edema.

Regulation of the temperature of the isolated perfused lungs

Most systems use temperatures of 37–38 °C [15,25]. Some authors tend to have the blood perfused preparations at a lower temperature (32–36 °C) [8]. It should be noted that in most systems where tubing is not in a heated environmental box, the temperature of perfusate increases after circulation is stopped. We switch off the heating during the setup procedure and start heating only after the demanded rate of perfusate flow is achieved.

Construction of the external perfusion circuit

It was reported that several materials used for the construction of the setup for the perfusion of isolated organs may have toxic effects, or may absorb chemicals in the circuit (for review see [8,9]). Care must be taken when choosing the brand of tubing and plastic connectors to be used. For isolated rat lungs we have had the best experience with the tubing obtained from usual transfusion sets (medical grade PVC, tubing approx. 4–5 mm o.d. with 0.5 mm wall thickness). They are not only suitable in size, but also in our hands free from adverse effects.

The cleaning of the external circuit is a very important procedure. First, all connections must be disconnected and all parts should be washed in 0.9% of NaCl. Then the tubing should be washed through with dilute sodium bicarbonate. All the parts must be cleaned mechanically by brush, the cannulae by pipe cleaners, and plastic tubing by rubbing them between the fingers. Protein residue which remains in the circuit may substantially affect the reactivity of the preparation. Then the tubing is left in the detergent solution overnight. Before the experiment, we wash through the tubing and all other parts of the setup with running tap water (20 min). Then after the circuit is put together, we flush it several times with distilled water, and finally with saline.

Viability of isolated perfused lungs

Isolated rat lungs perfused with physiological salt solution are stable even in long experiments lasting several hours [24,26]. The most common indicator of deterioration is an increase of

perfusion pressure which is often linked to imminent lung edema. The development of lung edema can be monitored by continuous recording of the weight of the preparation [14] or by the assessment of the ratio of the dry to wet weight of the preparation which should be less than 0.16 [25,27]. Useful indicators of lung injury are methods which determine changes in alveolo-capillary permeability [18], pulmonary capillary pressure [28] or the ability to transport oxygen from the alveoli into the perfusate [14].

Contribution of preparation to understanding the pulmonary vascular pathophysiology

The important benefit of the method is that main regulatory determinants of pulmonary blood vessels are controlled. Studies on isolated lungs of rats exposed to chronic hypoxia brought insight into mechanism of lung hemodynamic changes in hypoxic pulmonary hypertension [20]. Measurements on saline perfused rat lungs reported unexpected low basal production of NO which increased after exposure to chronic hypoxia [29]. The relative simplicity of preparation was found very useful in search for substances suitable for vasodilatory therapy of pulmonary hypertension [4].

Conclusion

Isolated perfused murine lung is experimental method suitable for studies on lung hemodynamic and metabolism. It is useful for analyses of acute and chronic increase in pulmonary vascular resistance and for experimental studies on treatment of pulmonary vascular diseases.

Acknowledgements

This work is supported by Grant GACR 305/08/0108, Cardiovascular Research Centre 1M510. Authors appreciate the help of Professor Martin Vízek with corrections of the manuscript.

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