Measurement of Extravascular Lung Water by the Double Indicator Dilution Method Using Heat and Sodium in Horses under General Anesthesia

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ABSTRACT. Rapid infusion is believed to be harmful to the lung, however, the pathological status of pulmonary edema resulting from excessive fluid therapy in horses has not been clarified because the quantitative diagnosis of pulmonary edema is impossible. To evaluate the precision of the double indicator dilution method using heat and sodium in horses, which allows the quantitative diagnosis of pulmonary edema, we compared extravascular lung water volume measured using a lung water computer based on the theory of the double indicator dilution method with that determined by the direct method. The value of extravascular thermal volume (ETV) determined by the double indicator dilution method was 7.82 ± 0.62 ml/kg and the detection ratio of ETV to the value of pulmonary extravascular water volume (PEWV) by the direct method was 0.996 ± 0.038. There was a significant correlation between ETV and PEW (P<0.05), and the regression line was Y=1.23 X − 1.73 with a correlation coefficient of 0.953. The value of extravascular lung water determined by the double indicator dilution method was significantly consistent with that obtained by the direct method, indicating the high precision of the double indicator dilution method in normal horse lungs. — KEY WORDS: double indicator dilution method, equine, extravascular lung water, extravascular thermal volume, general anesthesia.


Pulmonary edema is defined as a pathologic state in which extravascular lung water increases extraordinarily [30]. This disease can roughly be classified pathogenically as "hydrostatic pulmonary edema" due to increased pulmonary microvascular pressure and decreased plasma oncotic pressure, or "hyperpermeability pulmonary edema" due to increased pulmonary capillary permeability [15]. In horse, cases of suspected hyperpermeability and hydrostatic pulmonary edema have been reported following intensive fluid therapy for endotoxic shock with hemorrhagic colitis syndrome, and during anesthesia for enterotomy in horses with severe circulatory disturbance, respectively [1, 21, 32]. Thoracic radiography, often used as a diagnostic method for pulmonary edema, is not applicable to the quantitative diagnosis of pulmonary edema due to fluid therapy in horses [7], because they are too large to make an accurate image diagnosis by thoracic radiography [3, 4].

In recent years, the determination of extravascular lung water has been used, in humans and small animals, to establish a diagnosis of pulmonary edema more rapidly and objectively than thoracic radiography [2, 11]. The double indicator dilution method using heat and sodium as indicators, is considered the best method and its usefulness has been reported elsewhere [8, 12, 19, 26, 27].

To evaluate the precision of the double indicator dilution method using heat and sodium in horses, we compared the values of extravascular lung water measured using a lung water computer based on the theory of the double indicator dilution method with the values determined by the direct method.

MATERIALS AND METHODS

Experimental horses: Five adult thoroughbred horses with an average body weight of 449 (436–460) kg in which auscultation, electrocardiogram, and blood examinations revealed no abnormality were used in these experiments.

Anesthesia: The experimental horses were fasted for 8–12 hr before anesthesia but allowed free access to water. After sedation with xylazine (1.0 mg/kg body weight [BW]), general anesthesia was induced by a 10 per cent solution of guaifenesin (500 ml) and thiopental (4.0 mg/kg BW). The horses were held in a right lateral recumbent position, and anesthesia was maintained by inhalation of isoflurane and oxygen using a large animal anesthetic machine and ventilator (LAVC, 2000, J. D. Medical, U.S.A.). The arterial partial pressure of carbon dioxide (PaCO2) was maintained at 40 ± 5 mmHg and the concentration of expired isoflurane at 1.5 ± 0.1% by intermittent positive pressure ventilation. The duration of anesthesia was 2 hr and no drugs other than isoflurane were administered while the horses were anesthetized. Blood was anaerobically collected from the facial artery and the arterial blood gas pressures were immediately measured by a blood gas analyzer (ABL620, Radiometer, Denmark). The concentration of expired isoflurane was determined by an anesthetic gas monitor (Type1304, Bulel Care, Denmark).

Determination of the extravascular thermal volume (ETV) by the double indicator dilution method: From two introduces (8F, Terumo, Japan) placed in the left jugular vein, a catheter for indicator infusion (7F, 100 cm, Interbec catheter, Fuji systems, Japan) and a Swan-Ganz catheter (7F, 110 cm, SP5107, Viggo Spectramed, Singapore) were
inserted and the points of the catheters were positioned in the right atrium and the pulmonary artery, respectively, while monitoring pressure waveforms with a pressure transducer (DX-312, Viggo Spectramed, Singapore). Next, a lung water catheter (HE-2900, Viggo Spectramed, Singapore) was inserted from an introducer (5F, Terumo, Japan) placed in the left common carotid artery, and was positioned about 80 cm-distally to the initial part of the aorta in the left common carotid artery.

For the determination of ETV, 35 ml of 5% saline at 0°C was injected into the right atrium through the catheter for indicator infusion within 2 sec, using an auto injector (M800, Nemoto Kyorindo, Japan). Mean transit time of heat (MTT thermo) and mean transit time of saline (MTT saline) were measured with a Lung Water Computer (MTW1100, Nihon Koden, Japan) connected to the lung water catheter located in the left common carotid artery. Simultaneously, cardiac output was determined with a thermocardiographic output computer (2G82, NEC Sanei, Japan) connected to the Swan-Ganz catheter. The measurement with the Lung Water Computer and the thermocardiographic output computer were consecutively performed four times and the average value of the second through fourth measurements was computed. ETV was calculated using the following equation [12].

$$\text{ETV} = \text{Cardiac output} \times (\text{MTT thermo} - \text{MTT saline})$$

**Determination by the direct method:** Pulmonary extravascular water volume (PEWV) was determined by modifying Levine’s [13] and Pearce’s [20] methods, as the direct method. The difference between pulmonary extravascular tissue weight (PETW) and its dry weight was determined. Immediately after the measurement using the Lung Water Computer, the horses were sacrificed by exsanguination and the lungs were totally resected from the hilum. Using a syringe and catheter (30 cm, 14 gauge), as much blood as possible remaining in the vessels was removed by aspiration through the hilum. After that, the weight of the isolated lungs was measured to obtain the PETW value.

To obtain the dry weight of the lungs, each lobe was divided into 10–18 pieces and dehydrated in a bath filled with ethanol for 24–48 hr. The dehydrated lung pieces were dried in an oven kept at 60°C to constant weight. The obtained constant weight was then taken as the dry weight.

### RESULTS

Table 1 shows the results of the measurements. The double indicator dilution method showed the ETV to be 7.82 ± 0.62 ml/kg BW, while the direct method showed PEWV to be 7.87 ± 0.80 ml/kg BW and PETW 9.53 ± 0.94 g/kg BW. The detection ratio of the direct method of ETV/PEWV was 0.996 ± 0.038, while that of ETV/PETW was 0.823 ± 0.034.

Figure 1 indicates the relationship between ETV determined by the double indicator dilution method and PEWV determined by the direct method. ETV and PEWV values are plotted on the horizontal and vertical axes, respectively. A significant correlation was observed ($P<0.05$): the regression line is $Y = 1.23 X - 1.73$ and the correlation coefficient is 0.953.

Figure 2 shows the gross appearance of both the left and right lungs immediately after isolation. In all horses, the dependent lung regions were congested with blood and discolored.

### DISCUSSION

Horses with hemorrhagic colitis syndrome require intensive fluid therapy with balanced electrolyte solutions because dehydration may progress rapidly [1, 22]. However, fluid therapy is considered to be a cause of pulmonary edema [32], because endotoxins can impair the endothelium of the pulmonary vasculature, resulting in increased vessel permeability [29, 33]. Anesthesia for surgery such as an enterotomy can also be associated with severe circulatory disturbances [28], which require intensive fluid therapy with balanced electrolyte solutions to control circulation. Clinical symptoms of suspected pulmonary edema are the retention of leaked fluid in the nasal cavity and marked edema in the upper respiratory tract [23, 32]. Fluid therapy is an important treatment for horses with impaired circulation, but rapid fluid infusion may damage the lungs [33]. In addition, the quantitative diagnosis of pulmonary edema is

| Table 1. Comparison of extravascular thermal volume (ETV) with pulmonary extravascular water volume (PEWV) and pulmonary extravascular tissue weight (PETW) |
|---------------------------------|---------------------------------|-----------------|------------------|------------------|
| Direct method                  | Double indicator method         | Ratio           |                   |
| PEWV (ml/kg)                   | PETW (g/kg)                     | ETV (ml/kg)     | ETV/PEWV         | ETV/PETW         |
| 7.87 ± 0.80                    | 9.53 ± 0.94                     | 7.82 ± 0.62     | 0.996 ± 0.038    | 0.823 ± 0.034    |

Values are expressed as mean ± SD, n=5.
clinically impossible in horses, and therefore, the \textit{in vivo} pathological state of pulmonary edema due to excessive fluid therapy is unknown. In order to evaluate the precision of determining extravascular lung water volume by the double indicator dilution method using heat and sodium, which allows a quantitative diagnosis of pulmonary edema, we compared values obtained by this method with those obtained by the direct method.

When the direct method of determining extravascular lung water is used, the treatment of residual blood in the lungs and how the isolated lungs are dried affects the obtained values. Pearce’s method [20] and its modified method, namely Noble’s method [16] are generally used on dogs. Pearce’s method measures the total weight of isolated lungs without removing the blood. The isolated lungs are homogenized with a fixed amount of water, and the hemoglobin and specific gravity of the homogenate are determined. After weighing a fixed volume of homogenate to obtain the wet weight, it is dried at about 80°C for 48 hr, then its dry weight is measured. Blood is treated in the same way to calculate the PEWV in milliliters. Noble’s method differs from Pearce’s method in that the measurements of the specific gravities of blood and homogenate are omitted, and in that no correction is made for the lung hematocrit. Also, when Noble’s method is used, PETW and PEWV are expressed in grams.

It is difficult to treat blood remaining in isolated horse lungs by following Pearce’s and Noble’s methods because they are large and weigh about 4 kg (about 1% of the body weight). Therefore, we treated residual blood in the lungs following the method developed by Levine \textit{et al.} [13]. In addition, preliminary experiments showed that the deep parts of isolated lungs, when dried by Pearce’s method, were not dried but rather denatured because the isolated lungs were too large. Accordingly, both the right and left lungs were divided into 10–18 pieces, dehydrated in ethanol, and dried in a oven at 60°C until a constant weight was obtained.

Ethanol not only causes dehydration but also removes fat which has a possible effect on the dry weight. However, given that pulmonary adipose tissue is localized in the interstitial spaces of the bronchioles [6] and that heat can diffuse through lipids in addition to water, we broadly interpreted lipid as a part of water and used this drying method.

The rationale behind using two indicators for the determination of extravascular lung water is that the concurrent administration of an indicator that can permeate the pulmonary capillary and diffuse thoroughly the interstitial spaces of the lung (heat) with an indicator that cannot diffuse outside the capillary (Na) allows the calculation of the volume of pulmonary extravascular spaces by the subtraction of the volume determined with the non-diffusible indicator from that determined with the diffusible indicator [11, 14]. The double indicator dilution method is, therefore, strongly dependent on blood flow, and is affected by circulatory kinetics and the sites measured [12].

To minimize the effect of posture on the pulmonary circulation in this study, we used the lateral recumbent position, because in this position the pulmonary artery pressure can be kept at a higher value than in the supine position [5, 25]. The concentration of expired isoflurane was maintained at 1.5 ± 0.1% (1.2 minimum alveolar concentration) to keep the effect of the anesthetic drug on the circulation constant [24]. Intermittent positive pressure ventilation [9] was applied to maintain the PaCO\textsubscript{2} at 40 ± 5 mmHg because hypercapnia increases blood catecholamines and affects the circulatory system [31]. As a result, congestive alteration was observed in the dependent lung.
regions of the right lateral recumbent position. This finding means that the blood flow on the pulmonary vascular bed was unbalanced between the non-dependent and dependent lung regions [10, 17, 18]. There was also a bias in the distribution of the indicators. Noble and Severyngh [16] reported that extravascular lung water is underestimated by about 20% in normal dogs because of a bias in the distribution of indicators. In this study, there was clear evidence of an imbalance in pulmonary blood flow between the non-dependent and dependent lung regions, suggesting that extravascular lung water was underestimated more in horses than in dogs.

On the other hand, the indicators were injected into the right atrium and detected in the common carotid artery in this study. The diffusible indicator heat could therefore diffuse through tissues (besides the pulmonary water) such as the right and left heart tissue and the common carotid artery, suggesting that the pulmonary extravascular water volume may have been overestimated [16].

Ishibe et al. [12] reported that ETV was 8.27 ± 1.55 ml/kg BW, PEWV 5.82 ± 1.19 ml/kg BW, the detection ratio of ETV to PEWV (ETV/PEWV) 1.44 ± 0.28, and that of ETV to PETW (ETV/PETW) 1.19 ± 0.22 in normal dog lungs. In dogs, the double indicator dilution method overestimated PEWV determined by the direct method, but the value of ETV was similar to that of PETW. One explanation for this similarity may be that heat diffusion is not limited only to extravascular lung water, and therefore, that ETV represents a volume that includes lung water and other tissues [12].

The ETV in our study was similar to the original object of determination, PEWV rather than PETW. As in dogs, the double indicator dilution method may have overestimated pulmonary extravascular water volume in horses. However, as mentioned above, the underestimation due to an imbalance in the pulmonary blood flow between the non-dependent and dependent lung regions may have canceled out this overestimation, resulting in smaller errors in the detection ratio in horses than in dogs.

The results of this study show that extravascular lung water volume determined by the double indicator dilution method, is significantly consistent with values obtained by the direct method when the PEWV ranges from 7 to 9 ml/kg BW. This suggests that the double indicator dilution method is highly precise in normal horse lungs.

REFERENCES


