**ORIGINAL RESEARCH**

# Cardiovascular Deconditioning and Venous Air Embolism in Simulated Microgravity in the Rat


Decompression illness results from the formation and growth of gas bubbles within the tissues and venous blood; e.g., venous air emboli (VAE). Associated symptoms can range from mild skin itching or simple joint pain to central nervous system involvement and even cardiovascular collapse. The pathophysiology of DCI may also affect the lungs as the venous bubbles obstruct the pulmonary microcirculation decreasing cardiac output (CO), increasing pulmonary vascular pressures and altering lung fluid balance. The preponderance of actual symptoms, however, involve limb pain that is presumably caused by extravascular bubbles.

The adaptation of the cardiovascular system to the microgravity environment (cardiovascular deconditioning) appears to be due in part to a cephalad fluid shift, and has been extensively studied in the conscious rat (22). Astronaut EVA’s have brought into question the potential relationship between cardiovascular deconditioning and hypobaric DCI. Compared with careful ground-based studies conducted in hypobaric chambers, there is a lower incidence of DCI in space during the astronaut EVA’s than would be predicted (25). These studies have established the predicted incidence of DCI expected, both in relation to the final altitude following a direct or staged decompression, and subsequent to washout of tissue inert gas (e.g., nitrogen) by oxygen prebreathing prior to decompression (14). In addition, other factors such as exercise, ambulation, time at altitude and individual susceptibility have been examined (1). The interaction between hypobaric decompression and the deconditioned cardiovascular system has not been previously evaluated. The purpose of this study was to assess pulmonary and hemodynamic effects of simulated microgravity and hypobaric decompression illness in the conscious rat.

Operationally important medical challenges of manned spaceflight include microgravity-induced cardiovascular deconditioning, total body calcium loss, and the risk of decompression illness (DCI) during extravehicular activity (EVA). Altitude DCI deserves particular attention because it is a preventable complication, but one that can have serious consequences if left unattended (26). Evaluation of the interaction between microgravity exposure and decompression illness is appropriate in view of plans for extended duration missions and an overall increase in the number of EVA’s required for construction of the Space Station. It is intended that such investigation will lead to a better understanding of the interplay between DCI and the human cardiovascular system in a microgravity environment.
METHODS

All experiments and procedures were approved by the Institutional Animal Care and Use Committee at The University of Texas Medical School at Houston. Six groups of rats were studied. Groups 1 and 2 were used to assess the hemodynamic effects of tail-suspension with and without venous air infusion, whereas groups 3–6 were used for biochemical analysis. Each animal served as its own control.

Cardiovascular Function (Groups 1–2)

Instrumentation: The rats were anesthetized with halothane, intubated and ventilated under isothermic conditions. A left parasternal thoracotomy was performed and the pericardium exposed and sectioned. A 20-MHz pulsed Doppler flow probe was then placed around the ascending aorta for cardiac output measurement. The displacement probe used to measure left ventricular wall thickening was sutured to the left ventricular wall and an 18-gauge thoracic drain was positioned until closure of the thorax.

The ultrasonic flow probe leads were tunneled to the dorsum of the neck for externalization and the surgical wounds closed. Polyvinyl catheters were placed into the abdominal aorta via the femoral artery and the jugular vein for measurement of arterial blood pressure and central venous pressure. The catheters were also tunneled to the neck dorsum. The lungs were reinfated under vacuum and the wounds were infiltrated with bupivacaine (0.5%) for post-operative analgesia. Antibiotic therapy (gentamycin 5 mg · kg⁻¹ IM) was initiated for a minimum of 5 ds. The animals were weighed daily and inspected for signs of infection.

Experimental protocols: Group 1, (n = 8) designated VAE, was exposed to 3 h of venous air infusion. We elected to use VAE given as a continuous infusion to simulate the prolonged bubbling that is reported with hypobaric decompression exposures used to simulate an astronaut EVA. In these cases, VAE are detected with Doppler ultrasound probes for periods lasting several hours during the period of decompression. Injecting venous gas very slowly in the rat simulated that particular feature of decompression effects. Although this technique is well established for a number of species (8), little data are available for the rat. After hemodynamic parameters stabilized, rats received the venous air infusions into the central venous catheter at a rate of 0.015 ml · kg⁻¹ · min⁻¹ for 180 min. Cardiovascular data were recorded at 15, 30, 45, 60, 120 and 180 min during venous air infusion and at 15, 30, 45, 60, and 120 min and 24 h post infusion.

Group 2 (n = 8), designated TS-VAE, experienced cardiovascular deconditioning by tail-suspension with a 30° head-down tilt. The tail suspension model has previously been described in detail (21) and is widely used by investigators to simulate the fluid shifts commonly observed upon exposure to microgravity. Briefly, rats were fitted with a flexible foam-tape cast applied to the proximal half of the tail. The cast was attached to a swivel and the rats were suspended in a 30° head-down tilt. This apparatus allowed the rats to rotate in a 360° arc using their forelimbs, and permitted free access to food and water in a light/dark cycled environment. Following 96 h tail-suspension, and while maintained in the suspended position, the animals were infused with venous air for 3 h as described above.

Pulmonary Measurements (Groups 3–6)

Group 3 consisted of normoactive controls; group 4, tail-suspension only; group 5, VAE only; and group 6, tail-suspension-VAE. The rats in groups 5 and 6 received venous air infusions, as described above. Immediately post infusion, the animals were anesthetized with pentobarbital sodium (50 mg IP). An arterial blood sample was collected anaerobically for blood gas and plasma protein analysis. The animals were euthanized by exsanguination and the lungs were removed for the following measurements.

Pulmonary edema measurement: The amount of blood-free extravascular fluid (edema) formation was measured using a modified method of Pearce (23) to account for the residual blood volume in the organ. The extravascular lung water (EVLW) was expressed as the extravascular fluid to dry weight ratio.
Data were analyzed using ANOVA with Dunnett's correction for the individual comparisons in the biochemistry studies. The cardiovascular data were analyzed using a 2-way ANOVA. Individual comparisons were analyzed using Student's t-test with Bonferroni correction. Significance was considered at p < 0.05.

RESULTS

Cardiovascular measurements: In the TS-VAE group, SVR increased significantly by 28% from the onset of tail-suspension and remained elevated throughout the venous air infusion (Fig. 1). In the VAE group, SVR increased significantly by 23% above baseline within 60 min of venous air infusion and remained elevated for 5 h. SVR in the VAE group returned to baseline within 6 h. CVP remained unchanged in both groups.

Cardiac output in the VAE group decreased by 26% by the end of the 180 min venous air infusion (Fig. 1). This was significantly greater than the TS-VAE group, whose CO decreased by 9%. In the TS-VAE group, however, tail suspension alone caused a 11% decrease (non-significant) in CO prior to the venous air infusion. Decreases in CO from baseline were significant at 1, 2 and 3 h of venous air infusion for both TS-VAE and VAE groups, versus control. CO returned to baseline within 24 h after venous air infusion. Arterial blood pressure was not significantly altered by either the tail-suspension or VAE. WT remained unchanged in response to tail-suspension or venous air infusion.

TS-VAE and VAE rats showed a similar response in...
heart rate to venous air infusion. HR decreased significantly by the first hour of air infusion and remained decreased until the end of the infusion. The differences between the two groups were not significant. In both groups, HR returned to baseline within 24 h (Fig. 2).

**Pulmonary measurements:** In the VAE group, lung wet-to-dry ratio was increased significantly as compared to the three other groups. TS-VAE rats also showed a significant increase in lung wet-to-dry ratio as compared to the control group. Tail suspension alone did not cause a significant increase in extravascular lung water. Arterial blood gas values, pH, PaO\textsubscript{2}, and PaCO\textsubscript{2} were not different from control value in any experimental group, although the decrease in PaO\textsubscript{2} and the increase in PaCO\textsubscript{2} seen in the VAE and TS-VAE groups were consistent with the pathologic pulmonary changes reported with DCI. Pulmonary compliance was unchanged in all experimental groups (Table I).

Total arterial white blood cell counts (WBC) were unchanged in all experimental groups (Table II, top). Pulmonary arterial blood white cell counts were significantly increased, however, in the VAE group versus controls, but neither the TS nor the TS-VAE groups showed significant differences from the control group. Pleural fluid white blood cell count was decreased in the VAE group versus the controls. This appeared to be a dilutional effect due to an increase in pleural fluid volume. BAL WBC counts were unchanged. Arterial blood neutrophil counts in both the TS-VAE and the VAE groups were significantly increased over control. Pulmonary and systemic arterial neutrophil counts were significantly increased in both the VAE and the TS-VAE groups versus the control group (Table II). Pleural fluid neutrophil counts were increased significantly in all experimental groups versus the control.

Pleural fluid protein concentration was increased in the VAE group versus the controls, but there was no significant change in either the TS or the TS-VAE groups. Plasma protein levels were unchanged (Table II, bottom). BAL protein was unchanged in both TS-VAE and in VAE rats (Table II).

**DISCUSSION**

Previous reports describing cardiopulmonary responses to significant decompression illness included hypotension, pulmonary hypertension, pulmonary edema, decreased lung compliance, hemococoncentration and hypoxemia (3,7,10,12,15). In similar conditions, Bove et al. observed increased systemic vascular resistance, tachycardia, increased central venous pressure and decreased cardiac output (3). Cardiovascular symptom expression and severity depend primarily on the extent of the decompression injury, and hence the degree of venous bubble formation. In our experimental design, the use of a venous air dose standardized by weight insured that the venous gas insult was uniform throughout the study population. Our findings demonstrate an attenuation in the response to both pulmonary edema formation and the decrease in cardiac output with simulated weightlessness and venous embolization (TS-VAE group).

### TABLE I. LUNG EDEMA, COMPLIANCE AND BLOOD GAS DATA

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TS</th>
<th>VAE</th>
<th>TS-VAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVLW (mL/kg)</td>
<td>4.18 ± 0.21</td>
<td>3.80 ± 0.41</td>
<td>6.02 ± 1.68*</td>
<td>4.78 ± 0.73*</td>
</tr>
<tr>
<td>Compliance</td>
<td>2.20 ± 0.22</td>
<td>2.10 ± 0.21</td>
<td>2.00 ± 0.08</td>
<td>1.90 ± 0.17</td>
</tr>
<tr>
<td>PaO\textsubscript{2} (mmHg)</td>
<td>89.90 ± 5.18</td>
<td>91.00 ± 8.83</td>
<td>77.20 ± 7.78</td>
<td>75.70 ± 16.86</td>
</tr>
<tr>
<td>PaCO\textsubscript{2} (mmHg)</td>
<td>44.90 ± 2.35</td>
<td>50.30 ± 3.75</td>
<td>47.20 ± 2.33</td>
<td>47.50 ± 5.80</td>
</tr>
<tr>
<td>pH</td>
<td>7.35 ± 0.02</td>
<td>7.25 ± 0.02</td>
<td>7.24 ± 0.04</td>
<td>7.27 ± 0.03</td>
</tr>
</tbody>
</table>

Data are represented as mean ± S.D.
* p < 0.05 compared with control.
§ p < 0.05 compared with TS-VAE.

EVLW = extravascular lung water ratio.

### TABLE II. CELL COUNTS AND PROTEIN DATA

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TS</th>
<th>VAE</th>
<th>TS-VAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial WBC</td>
<td>7920 ± 386</td>
<td>7831 ± 838</td>
<td>7975 ± 1448</td>
<td>6066 ± 1051</td>
</tr>
<tr>
<td>Pulmonary WBC</td>
<td>7970 ± 427</td>
<td>8903 ± 1074</td>
<td>13139 ± 1242*</td>
<td>10457 ± 1840</td>
</tr>
<tr>
<td>BAL WBC</td>
<td>674 ± 53</td>
<td>371 ± 61</td>
<td>638 ± 141</td>
<td>880 ± 185</td>
</tr>
<tr>
<td>Pleural WBC</td>
<td>54462 ± 5050</td>
<td>60133 ± 9019</td>
<td>20539 ± 3005*</td>
<td>46169 ± 10354</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>14.90 ± 1.34</td>
<td>29.60 ± 6.34</td>
<td>70.10 ± 2.99*</td>
<td>68.10 ± 6.28*</td>
</tr>
<tr>
<td>Arterial</td>
<td>11.27 ± 0.96</td>
<td>24.10 ± 5.17</td>
<td>55.10 ± 4.38*</td>
<td>52.95 ± 31*</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>0.68 ± 0.19</td>
<td>2.60 ± 1.66</td>
<td>3.20 ± 1.52</td>
<td>2.30 ± 0.75</td>
</tr>
<tr>
<td>BAL</td>
<td>1.63 ± 0.24</td>
<td>4.60 ± 0.54*</td>
<td>11.40 ± 0.32*</td>
<td>10.60 ± 1.09*</td>
</tr>
<tr>
<td>Pleural</td>
<td>48.30 ± 2.09</td>
<td>51.92 ± 1.66</td>
<td>50.45 ± 2.33</td>
<td>49.15 ± 2.85</td>
</tr>
<tr>
<td>Plasma WBC</td>
<td>7.08 ± 0.60</td>
<td>8.55 ± 0.50</td>
<td>20.06 ± 4.92</td>
<td>14.91 ± 8.72</td>
</tr>
<tr>
<td>BAL WBC</td>
<td>18.64 ± 1.14</td>
<td>16.25 ± 1.27</td>
<td>27.68 ± 0.97*</td>
<td>13.77 ± 2.81</td>
</tr>
</tbody>
</table>

WBC = white blood cells.
Data are represented as mean ± S.D.
* p < 0.05 compared with control.
Several mechanisms may be involved in the response of the deconditioned cardiopulmonary system to venous air embolization. The changes in cardiac output may be due either to fluid shifts or to a direct effect on myocardial function. The effects on the lungs include vascular distension, changes in volume, changes in ventilation to perfusion matching and potential changes in diffusing capacity (28). Additional factors may include mediator-induced changes in vascular tone and endothelial permeability (27), lymphatic recruitment (30), as well as hypoxic pulmonary vasoconstriction.

In the present study, there was a slight but non-significant decrease in cardiac output with tail suspension, a finding that in magnitude is consistent with other studies (4). This change could be expected to prolong recovery from a venous gas insult, possibly by decreasing pulmonary blood flow and thereby reducing the rate of gas embolus washout. However, venous air infusion produced an acute decrease in cardiac output in all animals, whereas in the TS-VAE group, the decrease in cardiac output was significantly less than the decrease experienced by the VAE alone group. It might therefore be inferred that even though both groups demonstrated approximately the same total decrease in cardiac output, the initial decrease experienced by the TS-VAE group occurred prior to the VAE and therefore may have allowed for compensatory changes to occur.

The result of a higher cardiac output in the TS-VAE group (after first hour of air infusion) relative to the VAE group may have allowed an increase in pulmonary perfusion enabling deeper penetration of the emboli into the pulmonary vascular tree. The emboli would thus block a smaller segment of the pulmonary vasculature and therefore obstruct less of the pulmonary blood flow. This could result in a lower pulmonary artery pressure and less edema formation. A higher cardiac output might also increase the diffusion of gas from the emboli into the surrounding blood by exposing the emboli to a greater volume of blood per unit time, resulting in a more rapid rate of resolution (20). This process might also be influenced by accumulation of protein and coagulation products at the blood-bubble interface (24). Measurement of left ventricular myocardial wall thickening fraction, an indicator of myocardial contractility, demonstrated no significant changes in systemic arterial and pleural protein values further suggest that the permeability of the microvascular and alveolar membranes were less affected in the tail-suspension VAE rats than the VAE alone.

Conclusions drawn from the present study are subject to certain limitations. It has been shown by other investigators that venous gas emboli will be distributed according to the gravity vector. However, Chang et al. reported that the size of the bubble, the speed of the blood flow and the size of the vessels do play a role. The authors found that, under most circumstances, the emboli followed the higher branch of a bifurcation (11). While the current model mimics the greater homogeneity in blood flow seen in microgravity, one would still expect a preponderance of emboli in the “upper” lobes. Additionally, the bubbles produced by venous air embolization are larger than DCI-induced bubbles, consequently some differences in pulmonary arterial occlusion patterns and possibly in surface area dependent processes such as cellular activation and lung fluid balance might be expected. Although the results of the present study reflect some cardiopulmonary changes, clinical symptoms of DCI involving limb pain are usually attributed to extravascular gas.

Several theories have been reported to explain the observed low incidence of hypobaric decompression illness during EVA. A decrease in the formation of micronuclei due to reduced stress on the muscles, tendons and ligaments of the joints as a consequence of reduced activity (exposure) in the microgravity environment has been described (25). Additional reasons may involve actual reporting discrepancies related to operational factors such as crew motivation, redirected attention, masking of subtle pain, misinterpreting actual symptoms or actual reluctance to report DCI (2). The results of the present study do suggest, however, that more than one process may be responsible for the decreased incidence of DCI (at least in terms of cardiopulmonary effects), including an overall increase in tolerance to the combined effects of simulated microgravity and decompression.
REFERENCES

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