COSMOS 2044 -Lung Morphology Study -Experiment K-7-28

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SUMMARY

There are no previous studies investigating the effect of microgravity exposure during spaceflight on lung tissue. We examined the ultrastructure of the left lungs of 5 Czechoslovakian Wistar rats flown on the 13 day, 19+ hr. Cosmos 2044 mission, and compared them to 5 vivarium and 5 synchronous controls at 1-g conditions, and 5 rats exposed to 14 days of tail-suspension. Within 10 minutes of sacrifice by decapitation, the lungs were removed and immersed in 3% glutaraldehyde in 0.1M phosphate buffer (total osmolarity of the fixative: 560 mOsm; pH = 7.4). The tissue stored at 5°C was transported to our laboratory where it was processed for light and electron microscopy.

No significant perivascular cuffing caused by interstitial edema was present in the tissue samples. Some of the flight, tail-suspended, and synchronous control rats showed alveolar edema, while vivarium controls did not. The pulmonary capillaries appeared to be more congested in the flight animals than in the other groups. This could be related to the increased hematocrit due to the microgravity exposure. In all 5 flight, 4 tail-suspended, and 3 synchronous rats, red blood cells (RBC) were present in the alveolar spaces. The RBC were either suspended free in the alveoli or observed lining the alveolar wall. The frequency of RBC lining the alveolar walls appeared greater in the dorsal (gravity non-dependent) than in ventral (gravity dependent) regions of the lung in these three animal groups. In 3 of the vivarium controls, very few RBC were found in the alveolar spaces. Intra-capillary fluid-filled vesicles were observed in the flight, tail-suspended and synchronous animals, but not in the vivarium controls. The formation of intra-capillary fluid-filled vesicles has been previously associated with pulmonary hypertension induced by high altitude exposure and mitral stenosis.

In conclusion, pulmonary hemorrhage and alveolar edema of unknown origin occurred to a greater extent in the flight, tail-suspended, and synchronous control animals, and in the dorsal regions of the lung when compared to the vivarium controls. The cause of these changes, which are possibly due to an increase in pulmonary vascular pressure, requires further investigation.

INTRODUCTION

COSMOS 2044 has provided us the first opportunity to examine lung tissue in animals that were exposed to 14 days of space flight, as well as in rats exposed to 14 days of tail-suspension. We also examined the lungs of the synchronous control rats which were exposed to similar acceleration, vibration, and environmental temperature profiles as the flight animals, and vivarium control rats which were maintained at 1-g conditions.

Limited information is available regarding the effect of microgravity on the respiratory system. Several functional aspects of the respiratory system, such as alveolar ventilation, pulmonary blood flow, and respiratory mechanics have all been shown to be exquisitely sensitive to changes in gravity (West, 1977; Glaister, 1977). Microgravity exposure in man may cause a cephalad shift in body fluid. Pulmonary blood flow and alveolar ventilation becomes more uniform in microgravity (Stone, 1965; West, 1977; Michels, 1978). An increase in acceleration (as is experienced in the Soviet's Biosatellite during launch and re-entry phases), accentuates the non-uniformity of pulmonary ventilation and blood flow (Glaister, 1977), as well as produce pulmonary interstital edema (Weidner et al., 1981). Thus, exposure to extreme gravitational forces, from 0-g (spaceflight) to 6-g (deceleration phase of re-entry) as occurred in both the flight and synchronous animals, may induce pathological changes in the lung related to abnormal lung fluid balance, altered pulmonary capillary hemodynamics and possible pulmonary hypertension.

Though small animals such as rats are not expected to undergo a major cephalad fluid shifts during microgravity exposure as does man, tail-suspension rats do experience large cephalad shifts in body fluids (Deavers et al., 1980). The fluid shift due to tail-suspension may be similar to that seen in man during head-down tilt, head-out-of water immersion and microgravity exposure (Arborelius et al, 1972; Blomqvist and Stone, 1985). In man exposed to these three experimental conditions, an increase in intrathoracic fluid and transient increase in pulmonary vascular pressures are seen (Arborelius et al, 1972; Katkov, 1983). Thus, the tail-suspension rat model gave us the opportunity to investigate the effects of increased intrathoracic fluid and pulmonary vascular pressure on lung ultrastructure.

This report summarizes our findings in the COSMOS 2044 flight, tail-suspension, synchronous

and vivarium controls. Our data, which are preliminary, suggest that microgravity, cephalad fluid shift and/or positive acceleration induced pathologic changes in the lung.

METHODS

The lungs from each of the five flight, tail suspension, synchronous, and vivarium animals were removed from the thoracic cavity within 10 minutes of decapitation. No precautionary measures were taken to ensure that aspiration of blood did not occur post-decapitation. The left lungs were immersed in glutaraldehyde (GA) fixative, (3% GA in 0.1M phosphate buffer total osmolarity of fixative: 560mOsm; pH = 7.4), and then transported to our laboratory at 5°C.

First, a 3-4 mm thick tissue slab was cut perpendicular to the cranio-caudal axis just across the most caudal aspect of the hilum. This tissue was embedded in paraffin. Sections (5-6 μm) were stained with hemotoxin-eosin stain and examined by light microscopy.

Samples for electron microscopy were then taken from the most ventral and dorsal aspects of the remaining lower lobe. A piece of lung tissue (approx. 2mm x 2mm x 4mm) was removed from each region and further divided into 1mm x 1mm x 2mm cubes. The tissue samples were rinsed overnight in 0.1M phosphate buffer adjusted to 350 mOsm with NaCl. They were post-fixed for 2 hours in 1% solution of osmium tetroxide in 0.125 M sodium cacodylate buffer adjusted to 350 mOsm with NaCl (total osmolarity: 400 mOsm, pH 7.4). They were dehydrated in increasing concentrations (70%-100%) of ethanol, rinsed in propylene oxide, and embedded in Araldite. One block was selected randomly from each site (dorsal/ventral). One micron sections were cut from each block using an LKB ultratome III. They were stained with 0.1% toluidine blue aqueous solution and examined by light microscopy. Ultrathin sections (50-70 nm) were contrasted with uranyl acetate and bismuth subnitrate (Riva, 1974) and examined with a Phillips 300 electron microscope.

All the paraffin embedded tissue was examined at magnifications of 100X and 400X for evidence of perivascular cuffing of the large pulmonary vessels and gross changes in the dependent/non-dependent lung regions. The 1μm sections were systematically examined at magnifications of 400X and 1000X (oil immersion) for peribronchial cuffing of smaller pulmonary vessels, presence of alveolar edema, and general appearance of the pulmonary capillaries and lung

parenchyma. The ultrastructure of the blood-gas barrier (capillary endothelium layer, interstitium space and epithelium layer) was examined by electron microscopy.

RESULTS

The preliminary COSMOS-2044 report by V.I. Korolkov (from the Institute of Biomedical Problems,Moscow, USSR) during the NASA/Ames Preliminary Results Symposium at San Jose, CA, 1990, indicated that when the animal were first examined at the recovery site, they appeared active and in satisfactory condition. By the onset of dissection, 5 hrs post-recovery, they were inactive and had reddish fluid drops on the tips of their nose. These signs were attributed to the gravitational stress which developed after the transition from 0-g to Earth's gravity. The re-entry profile consisted of approximately 3-5 minutes of 6-g deceleration phase, followed by 10 milliseconds of approximately 50-g during impact, and finally a return to Earth's gravity.

A reddish nasal discharge was not reported to occur in either the tail-suspended or synchronous control rats. However, it has been described to occur during the first few days post tail-suspension in rats (COSMOS 2044 Investigator, Dr. Daniel Riley, Medical College of Wisconsin, Expt. #K-7-09; personal communication). This nasal discharge, which has not been systematically investigated, may be associated with the stress response which occurs during the first few days of tail suspension (Thomason and Booth, 1990).

Light Microscopic Examination. The degree of lung inflation was not uniform within the tissue samples. All lung slabs tended to be centrally collapsed, while the periphery was moderately to well inflated. This could be due to the immersion fixation process since lung inflation was not controlled. No obvious evidence of perivascular cuffing was observed in any group. Red blood cells (RBC) were seen in the lumen of major airways in all samples, with the least observed in the vivarium animals. RBC could be seen in the airspaces of the centrally collapsed regions of the sections, as well as the periphery.

The vivarium control animals showed no evidence of alveolar edema in either the dorsal or ventral aspects of the lung (figure 1a). This tissue showed very few RBC in the alveoli, and the

pulmonary capillaries did not appear distended with RBC. No apparent dorsal/ventral gradient in the presence of RBC was detected in these control animals.

In the flight animals no evidence of perivascular cuffing was observed (Fig. 1b). Some evidence of alveolar edema was found in one out of five animals. It occurred in both the dorsal and ventral regions of the lung. A small number of RBC was observed in the alveoli of the flight tissue, except for one animal which showed a greater number RBC in the alveoli than the others. Overall, the dorsal sections generally showed more RBC in the airspaces than the ventral sections. RBC were seen in the alveoli (dorsal samples, 5 out of 5 animals; ventral, 4 out of 5). Densely packed RBC were found lining the alveoli (dorsal samples, 4 out of five; ventral, 2 out of 5). Finally, in 3 out of 5 animals, densely RBC packed pulmonary capillaries were observed in both the dorsal and ventral samples.

Tail-suspension in the rat is primarily used as a model for producing hind limb muscle atrophy and bone degeneration. It also can be used as a model for producing a cephalad fluid shift similar to that seen in humans exposed to microgravity, head-down tilt, and head-out of water immersion. Therefore, it was of particular interest to us for studying the effects of a cephalad fluid shift, and the associated possible increase in thoracic intravascular pressures on pulmonary ultrastructure. We found evidence of alveolar edema in both dorsal and ventral samples from 2 of the 5 animals. Four of the 5 tail-suspended animals also showed both RBC free in the alveolar space, as well as lining the alveoli (figure 1c). This generally occured to a greater extent in the dorsal than the ventral samples. Capillary RBC congestion was not apparent in this group of animals.

In the synchronous control group, 2 animals showed evidence of alveolar edema in the dorsal region of the lung. RBC were seen in the alveoli (dorsal/ventral samples) in 3 of the 5 animals Capillaries appeared moderately distended with RBC in two of the synchronous control animals.

Electron Microscopic Examination In the vivarium controls, the lung tissue appeared well preserved (Fig. 2a). The alveolar epithelium layer was smooth and intact with few cytoplasmic vesicles. The capillary endothelium appeared very thin on the alveolar side. This attenuation of the endothelial layer is commonly seen in rat pulmonary capillaries (Weibel, 1972). Several plasmalemmel vesicles

were seen on the interstitial side of the capillary. The basement membrane appeared fused along the thin side of the blood-gas barrier. It separated into epithelial and endothelial basement membranes at the transition to the thick portion of the blood gas barrier. The interstitial fibrous and collagen network appeared free of any excess interstitial fluid. Occasionally macrophages were observed lining the alveoli and in the interstitium.

In flight, tail-suspension and synchronous animals, RBC were seen densely packed along the epithelial lining of the alveoli (figure 2b) or singly in the alveolar space (Fig. 2d). Alveolar edema was observed in these three groups, as well as fluid-filled protrusions into the pulmonary capillary (figure 2c). The vesicles appeared to be formed by the invagination of the endothelium. They contained some granular material which appeared "incapsulated" by the endothelial layer. We interpreted this granular material as edema fluid. Such vesicles were not seen in the vivarium control animals. Complete epithelial disruption, and edematous blebbing was observed in one tail-suspended rat (Fig. 2d).

An increase in the number of alveolar macrophages occurred in some animals. This occured especially in one of the synchronous control rats where the macrophage population showed a large density of dark cytoplasmic lysosomes occlusions (Fig. 2c), suggesting an increase in the protolytic activity of the cells.

DISCUSSION

The major finding of this study is the evidence of pulmonary hemorrhage of unknown origin, and the abnormality of pulmonary fluid balance in all three treatment groups: flight, tail-suspension and synchronous animals as compared to the vivarium controls.

Limitations and technical problems. An obvious limitation in this study is the immersion fixation of the lung. Understandably, optimal fixation of the lung tissue by vascular perfusion *in situ* could not be performed since it was incompatible with the collection of several other tissues. Immersion fixation provided us with an effective means of tissue preservation, but it did not allow us to control a uniform degree of lung inflation for a quantitative comparison of the blood-gas barrier thickness

and/or the degree of perivascular cuffing between groups. In spite of this limitation, we were successfully able to qualitatively describe the ultrastructure of the lung parenchyma in each animal group.

A second limitation could be the fact that no precautionary measures (such as clamping the trachea immediately post decapitation) were taken to prevent possible aspiration of blood by the animal post-decapitation. Indeed, it clouds our interpretation of the etiology of the pulmonary hemorrhage seen in flight, tail-suspension and synchronous animals. However, we believe that the origin of the observed alveolar hemorrhage is not solely due to the aspiration of blood, since the vivarium animals sacrificed in the exact same manner as the other animals showed far fewer RBC, if any, in the alveolar space.

Findings. Pulmonary hemorrhage has been shown to occur in response to a severe elevation in pulmonary vascular pressures during exposure to extreme high altitude (reduced ambient oxygen tension), severe chronic mitral stenosis disease, and in neurogenic pulmonary edema. In all these cases, extravasation of RBC outside the pulmonary capillary have been observed (Cottrell et al., 1967; Heath et al., 1973; Kay et al., 1973; Minnear et al., 1987; Bachofen et al., 1988). Very recently we systematically investigated the effect of increased capillary transmural vascular pressure on lung ultrastructure. We consistently found structural disruptions of the blood-gas barrier, with RBC squeezing into the interstitial and alveolar spaces at capillary transmural pressures greater than 52.5 cm water (Tsukimoto et al., 1990). It is possible that exposure to different gravitational forces, as occurs during tail-suspension, positive acceleration, and/or during the readaptation to Earth's gravity may induce a transient elevation in pulmonary vascular pressure and possible pulmonary hemorrhage.

Although conclusive characterization of the exact etiology of the pulmonary hemorrhage is not possible at this point, it is noteworthy that the appearance of RBC in the alveolar spaces occurred to a greater extent in the flight, tail-suspended, and synchronous animals than in the vivarium controls. The slightly greater occurrence of hemorrhage in the dorsal regions could reflect a difference in the vulnerability of the capillaries in that region, in spite of the limited dorsal/ventral gravity dependent gradient expected to occur in such a small animal as the rat.

Other evidence that suggest that the flight, tail-suspended and synchronous rats may have been exposed to either chronic or transient pulmonary hypertension is the finding of disrupted epithelial layers of the blood gas barrier, and the presence of intra-capillary endothelial vesicles. Both these abnormalities have been associated with hemodynamically induced pulmonary edema. In the study by Tsukimoto et al. (1990), disruptions of the epithelial layer of the blood-gas barrier were consistently observed at capillary transmural pressures of 52.5 cm water and above. Minnear et al. (1981) also showed evidence of epithelial disruptions after animals were transiently exposed to severe pulmonary hypertension caused by experimentally induced neurogenic pulmonary edema. Strikingly similar vesicle formation has been observed in rats exposed to simulated high altitude conditions (Heath et al., 1973) and in humans with chronic mitral stenosis and pulmonary hypertension (Kay and Edwards, 1973). Intra-capillary vesicles have also been observed in permeability induced pulmonary edema. Toxic agents, such as monocrotaline, ammonium sulphate and alpha-naphthylthiourea, used to induce acute pulmonary edema in experimental animals were found to induce similar vesicle formation in the pulmonary capillary (Valdivia et al., 1967; Kay et al., 1969; Hayes et al., 1970; and Meyrick et al., 1972). These substances are believed to cause a permeability type of pulmonary edema, by inducing toxic changes in the endothelial and epithelial layers causing fluid to accumulate in the interstitial space on the thin side of the blood gas barrier, thus inducing separation and evagination of the endothelial layer forming edema filled vesicles. The flight, tail-suspension and synchronous animals which showed intra-capillary vesicles probably experienced pulmonary hypertensive episodes which could have induced a hemodynamic form of pulmonary edema.

Further studies are clearly needed to characterize the etiology of the structural changes observed in the lungs of the COSMOS 2044 rats. These studies include the analysis of the effect of microgravity exposure on lung structure in other groups of rats, such as in SLS-1 experiments, as well as the systematic analysis of lungs by vascular perfusion in tail-suspended animal.

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REFERENCES

Arborelius, M., U.I. Balldin, B. Lilja, and C.E.G. Lundgren. Hemodynamic changes in man during immersion with the head above water. *Aerosp. Med.* 43:592-598, 1972.

Bachofen, H., M. Bachofen, and E.R. Weibel. Ultrastructural aspects of pulmonary edema. *J. Thorac. Imag.*, 3(3):1-7, 1988.

Blomqvist, C.G., and H.L. Stone. Cardiovascular adjustments to gravitational stress. *Handbook of Physiology*, Sect. 2. The Cardiovascular System. Vol. III. Edited by J. T. Shepard and F. M. Abboud, Bethesda, American Physiological Society, pp. 1025-1063. 1985.

Cottrell, T.S., O.R. Levine, R.M. Senior, J. Wiener, D. Spiro, and A.P. Fishman. Electron microscopic alterations at the alveolar level in pulmonary edema. *Circulat. Res.*, 21:783-797, 1967.

Deavers D.R., X.J. Musacchia, and G.A Meininger. Model for antiorthostatic hypokinesia: head-down tilt effects on water and salt excretion. *J. Appl. Physiol.* 49(4): 576-582, 1980.

Glaister, D.H., Effect of acceleration In: West, J.B. ed. *Regional differences in the lung* New York: Acedemic Press, 1977.

Hayes, J.A., and A. Shiga. Ultrastructural changes in pulmonary oedema produced experimentally with ammonium sulphate. *J. Pathology*, 100: 281, 1970.

Heath, D., H. Moosavi, and P. Smith. Ultrastructure of high altitude pulmonary oedema. *Thorax*, 28: 694-700, 1973.

Katkov, V.E., V.V. Chestukhin, E.M. Nikolayenko, V.V. Rumyantsev, and S.V. Gvozdev. Central circulation of a normal man during 7-day head-down tilt and decompression of various body parts. *Aviat. Space Environ. Med.* 54(Suppl. 1): S24-S30 (1983).

Kay, J.M., P. Smith, and D. Heath. Electron microscopy of *Crotalaria* pulmonary hypertension. *Thorax*, 24:639, 1969.

Kay, J.M., and F.R. Edwards. Ultrastructure of the alveolar-capillary wall in mitral stenosis. *J. Pathology*, 111:239-245, 1973.

Meyrick, B., J. Miller, and L. Reid. Pulmonary oedema induced by ANTU, or by high or low oxygen concentrations in the rat-an electron microscopic study. *Br. J. Exp. Path.*, 53:347, 1972.

Michels D.B., and J.B. West. Distribution of pulmonary ventilation/perfusion during short periods of weightlessness. *J. Appl. Physiol.*, 45:987-998, 1978.

Minnear F.L. and R.S. Connell. Increased permeability of the capillary-alveolar barriers in neurogenic pulmonary edema (NPE). *Microvasc. Res.* 22:345-366, 1981.

Minnear F.L., C. Kite, L.A. Hill, and Hoyte VAN DER Zee. Endothelial injury and pulmonary congestion characterize neurogenic pulmonary edema in rabbits. *J. Appl. Physiol.* 63(1): 335-341, 1987.

Riva, A. A simple and rapid staining method for enhancing the contrast of tissue previously treated with uranyl acetate. *J. Microsc.* (Paris). 19:105-108, 1974.

Stone, H.L., B.H. Warren and H. Wagner. The distribution of pulmonary blood flow in human subjects during zero-G. AGARD *Conf. Proc.*, 2:129-148, 1965.

Thomason, D.B. and F.W. Booth. Atrophy of the soleus muscle by hindlimb unweighting. *J. Appl. Physiol.*, 68(1): 1-12, 1990.

Tsukimoto, K., O.Mathieu-Costello, R. Prediltto, A.R. Elliott, and J.B. West. Ultrastructural appearances of pulmonary capillaries at high transmural pressures. *J. Appl. Physiol.* (submitted), 1990.

Valdivia, E. R., J.J. Lalich, Y. Hayashi, and J. Sonnad. Alterations in pulmonary alveoli after a single injection of monocrotaline, *Archs. Path.*, 84:64, 1967.

West J.B. ed. *Regional differences in the lung.* New York: Acedemic Press, 1977.

Weibel, E.R., Morphometric estimation of pulmonary diffusing capacity. V. Comparative analysis of mammalian lungs. *Resp. Physiol.*, 14:26, 1972.

Weidner W.J., L.F. Hoffman and D.O. Defouw. Effect of sustained Gz acceleration on lung fluid balance: An ultrastructural Study. *Physiologist*, 24(6), S85-S86.