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Hypergravity Alters the Susceptibility of Cells to Anoxia-Reoxygenation Injury

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INTRODUCTION: Gravity is a physical force, much like shear stress or mechanical stretch, and should affect organ and cellular function. Researchers have shown that gravity plays a role in ventilation and blood flow distribution, gas exchange, alveolar size and mechanical stresses within the lung (1-3). Short exposure to microgravity produced marked alterations in lung blood flow and ventilation distribution while hypergravity exaggerated the regional differences in lung structure and function resulting in reduced ventilation at the base and no ventilation of the upper half of the lung (4). Microgravity also decreased metabolic activity in cardiac cells, WI-38 embryonic lung cells, and human lymphocytes (5). Rats, in the tail-suspended head-down tilt model, experienced transient loss of lung water (6), contrary to an expected increase due to pooling of blood in the pulmonary vasculature. Hypergravity has also been found to increase the proliferation of several different cell lines (e.g., chick embryo fibroblasts) while decreasing cell motility (7) and slowing liver regeneration following partial hepatectomy (8). These studies show that changes in the gravity environment will affect several aspects of organ and cellular function and produce major change in blood flow and tissue/organ perfusion. However, these past studies have not addressed whether ischemia-reperfusion injury will be exacerbated or ameliorated by changes in the gravity environment, e.g., space flight. Currently, nothing is known about how gravity will affect the susceptibility of different lung and vascular cells to this type of injury. We conducted studies that addressed the following question: Does the susceptibility of lung fibroblasts, vascular smooth muscle and endothelial cells to anoxia/reoxygenation injury change following exposure to hypergravity conditions?

EXPERIMENTAL METHODS: Bovine aorta endothelial (BAEC) and primate smooth muscle(SMC) were obtained from the NIGMS/Coriell Cell Repository. Rat lung fibroblasts (RFL) were isolated from adult Sprague Dawley rats as previously reported (9). All cell lines were maintained in DMEM containing 10% fetal bovine serum. Confluent cultures of each cell line were subjected to centrifugation at 6G for 24-48 hrs. Control cultures were not centrifuged or rotated. Cells were then placed under anoxia (5% CO_2 /balance N_2) for 2 hr with or without a 1 hr period of reoxygenation. The change in viable cell numbers was assessed by: measuring viable cells by hemacytometer counting of trypan blue stained cells, or using MTT assay for viable cells (microtiter plate assay). The effect of hypergravity on the expression of heat shock protein (HSP60) by RFL was evaluated by immunocytochemical staining using a FITC-labeled monoclinal antibodies (Stress Gene). Subconfluent cultures were fixed after 12 and 24 hr under hypergravity using 10%formalin, 0.1 % triton X-100 in PBS. Cultures were incubated with the primary antibody for 1 hr, washed 3X with PBS, incubated with biotin labeled anti IgG secondary antibody for 1 hr and subsequently stained with fluorescein conjugated streptavidin. Cells were viewed by fluorescence microscopy and photographed. Controls, stained with non-immune mouse IgG, showed no fluorescence under these conditions.

RESULTS AND DISCUSSION: Figure 1 shows the change in viable cell count, expressed as percent of controls, for SMC and BAEC as a function of time under 6G. The solid line at 100°/0 represents the level for cultures that were not treated with anoxia or anoxia-reoxygenation. SMC were found to have a 60°/0 decrease in viable cells after 48 hr of hypergravity and 2-hr of anoxia. In the first 24 hr of hypergravity, the acute response of SMC seems to be an exacerbated injury under anoxia and reoxygenation. This acute phase is followed in the next 24 hr by a reversal of the exacerbated injury as cells adapt to hypergravity.

The results found for BAEC show a different pattern. Control (non-centrifuged) cells had decreased viability under anoxia with only a slight further decrease during the reoxygenation period. With BAEC, the acute response (first 24 hr) to hypergravity is an increase in viable cells under anoxia-reoxygenation. As seen with SMC, there is a reversal of this increase in the following 24 hr period

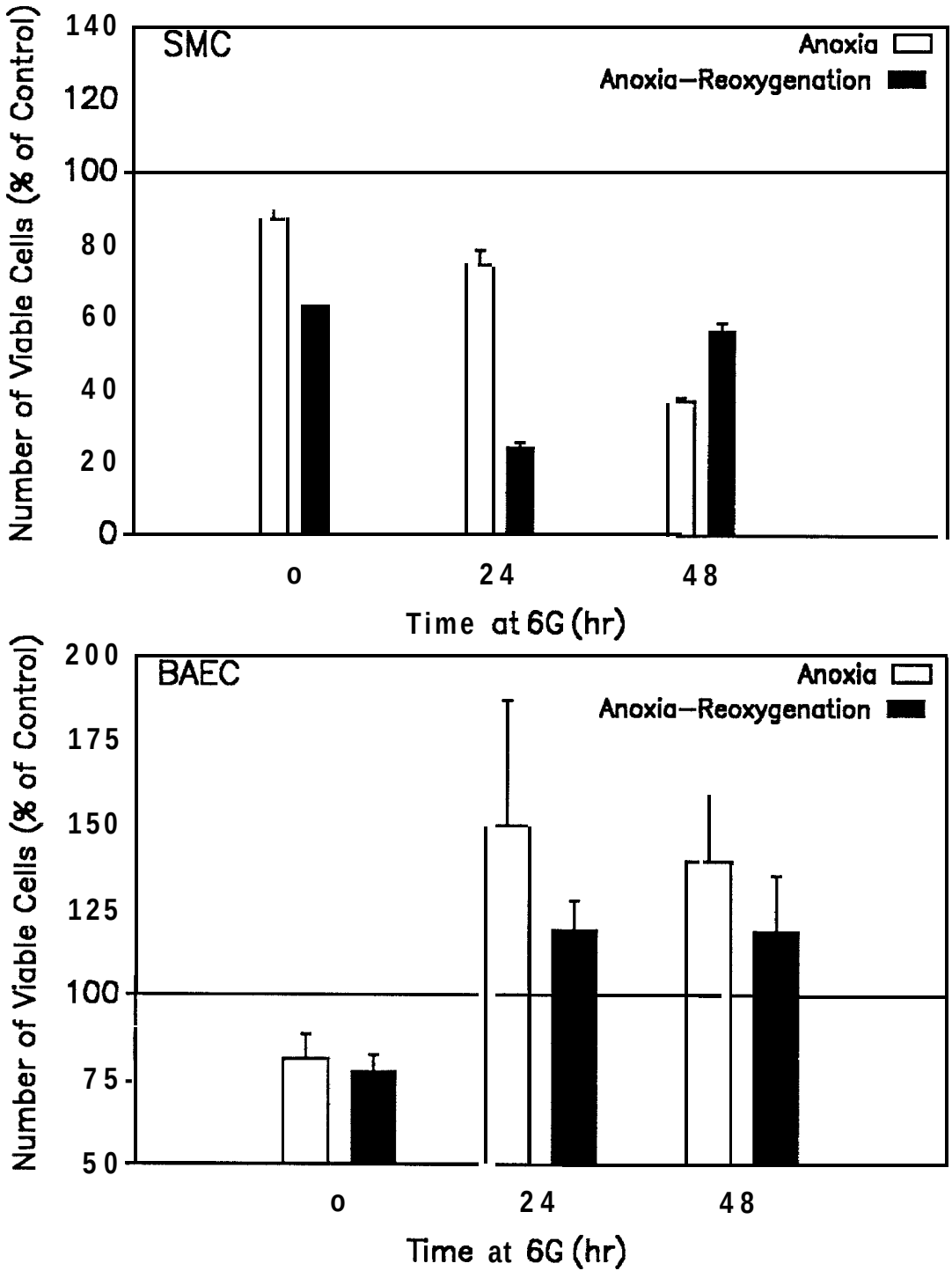

FIGURE 1. The response of SMC and BAEC to anoxia or anoxia-reperfusion injury following hypergravity treatment for 24-48 hr. Data is plotted as mean of the % of matched untreated (controls) normogravity or hypergravity cultures.

as the cells adapt to hypergravity. Although it is expected that more cell damage and loss of viability would result from the combined effect of anoxia and reoxygenation, we found that 2 hr of anoxia results in similar damage. Hypergravit y worsened the damage for SMC but decreased the damage for BAEC, suggesting that protective mechanisms maybe differentially activated in vascular cells.

Figure 2 shows that results similar to those seen with BAEC were also found for RFL whether these cells were maintained in DMEM or PBS. When maintained in complete media (DMEM), these cells show an increase in viable cells with time in hypergravity when treated with anoxia alone. However, RFL maintained in PBS show a steady decrease with time in hypergravity. RFL behave similar to BAEC when placed under anoxia-reoxygenation. The acute response of RFL to hypergravity is a transient decrease in susceptibility to damage, i.e., increased viable cell counts, under anoxia-reoxygenation. Again this is followed by a reversal in the following 24 hr period as the cells adapt to hypergravity. This pattern of response was also found for RFL maintained in PBS.

When BAEC and RFL have apparently adapted to hypergravity, both cell lines are not readily injured by anoxia-reoxygenation. In fact, RFL maintained in PBS, which has no energy source (e.g., glucose) was not found to have the maximum injury under hypergravity as expected. Under these conditions RFL responded similarly to cells in complete media. These results suggest that hypergravity may increase protective mechanisms in these cells that ameliorate possible damage from anoxia-reoxygenation. One possible protective mechanism that maybe induced by hypergravity is increased expression of stress proteins like heat shock proteins. Heat shock proteins have been shown to protect cells from damage and death under a number of different stresses, e.g., increased temperature or sheer stress (10). We tested this possibility with RFL, as shown in figure 3. Within 12 hr under hypergravity, RFL's had clearly increased immunofluorescence for heat shock protein, HSP60 (figure 3A) compared to control cells (figure 3 C). By 24 hr, hypergravity resulted in an even more intense immunofluorescence, suggesting that HSP60 expression is stimulated for at least the initial 24 hr period. Preliminary data from similar studies with BAEC (data not shown) indicate that these cells also may increase their expression of heat shock proteins within the first 24 hr period of hypergravity. Further studies are planned to examine the possible mediation of hypergravity effects on cells by the heat shock protein family.

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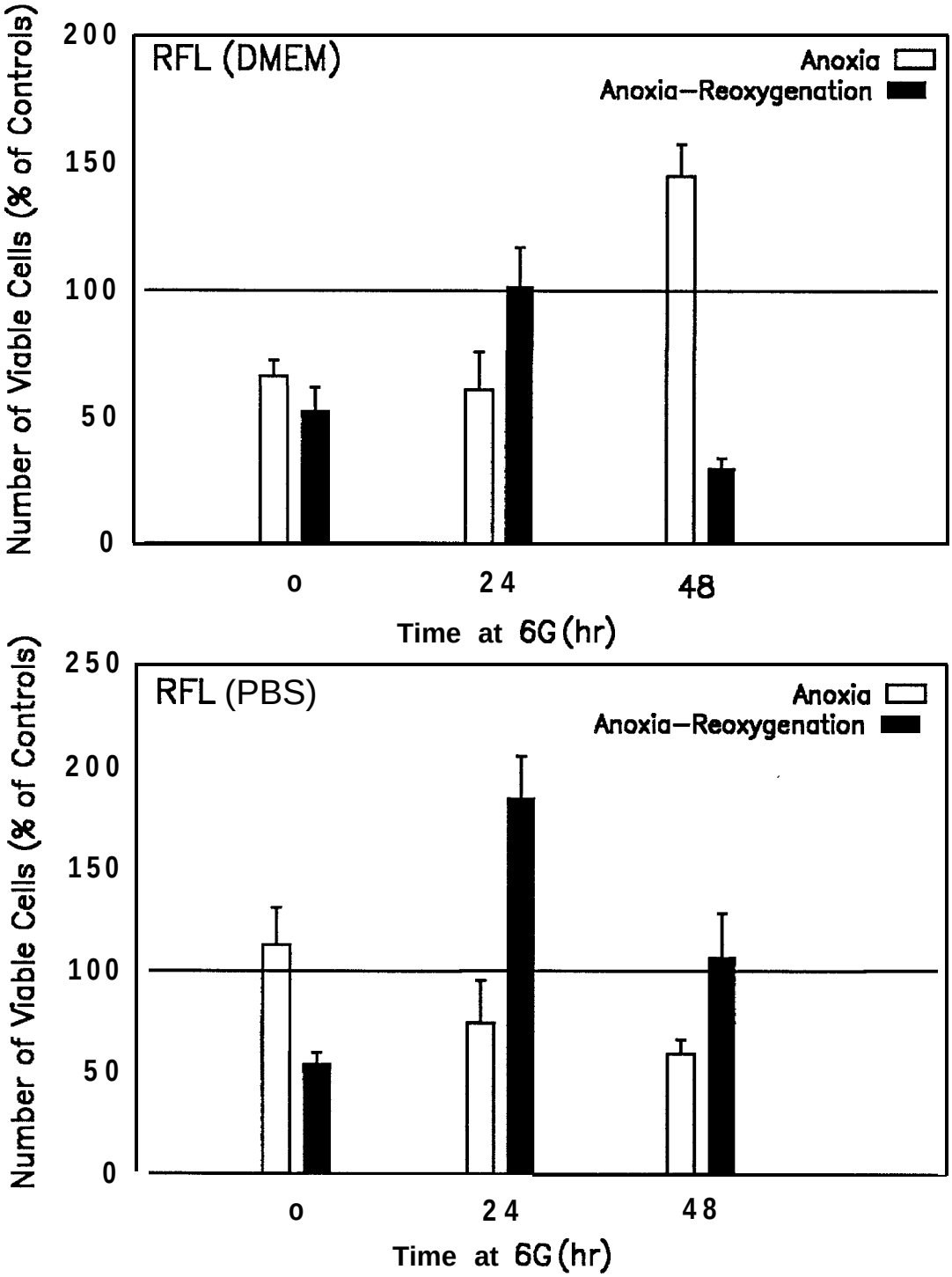

FIGURE 2. The response of RFL, maintained in complete media (DMEM) or in PBS, to anoxia or anoxia-reperfusion injury following hypergravity treatment for 24-48 hr. Data is plotted as mean of the 1%0 of matched untreated (controls) normogravity or hypergravity cultures.

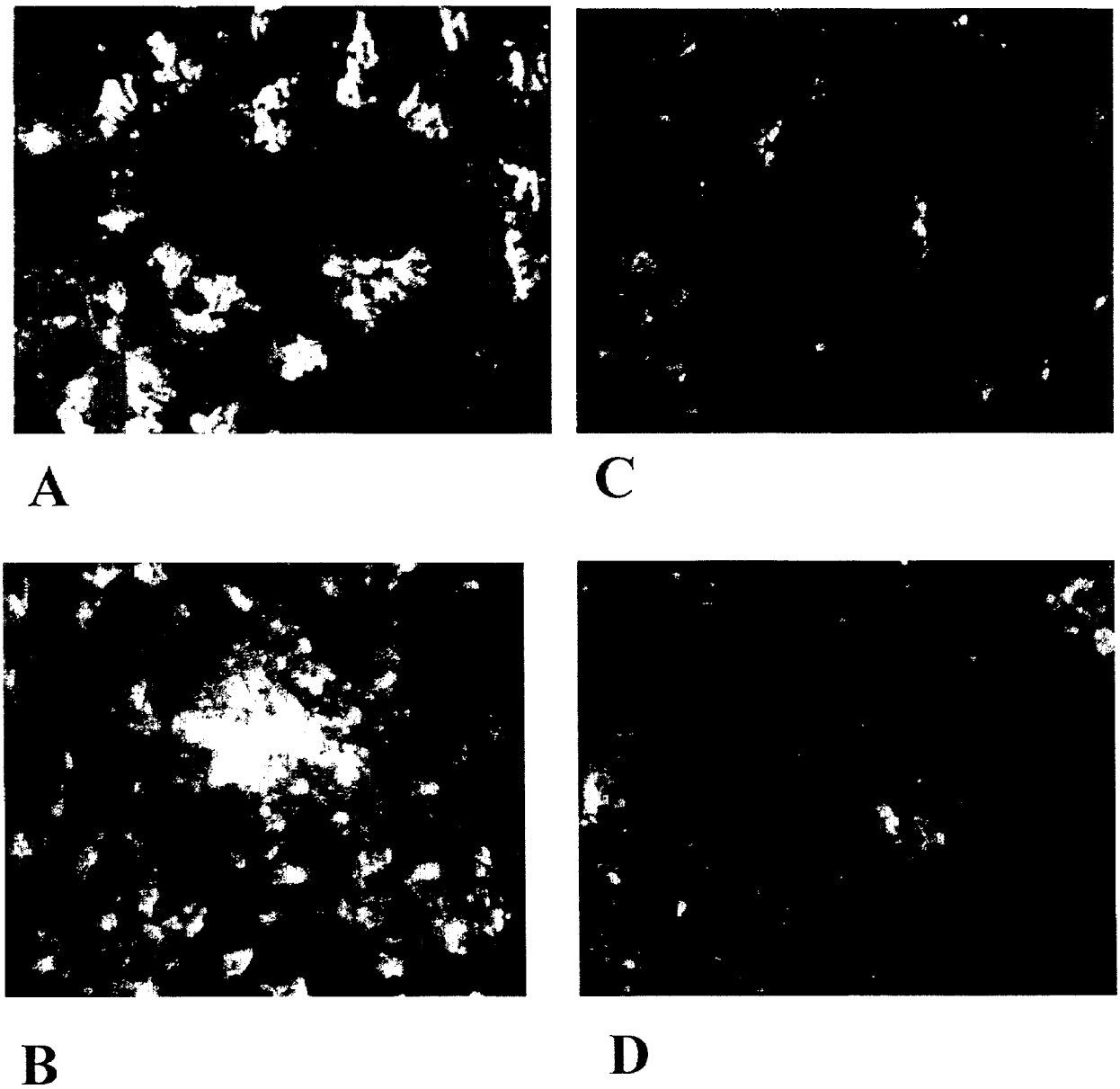

Figure 3. Expression of heat shock protein (HSP60) by RFL subjected to 12 and 24 hr of h ypergravity (A & B) or normogravity (C & D).