ERYTHROPOIETIN AND IL-3 RECEPTOR CELL SURFACE EXPRESSION IS DECREASED UNDER CONDITIONS THAT MODEL SOME ASPECTS OF MICROGRAVITY

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Human and experimental animals in space develop the anemia of space flight (Tavassoli, M., 1982; Udden et al., 1995). Studies have shown that erythropoietin (Epo) is implicated in this microgravity-induced abnormality (Udden et al., 1995). The status of the Epo receptor (EpoR) in microgravity, however, is unknown. The Rotary Cell Culture System (RCCS), based on NASA rotating wall vessel (RWV) technology and manufactured by Synthecon Inc., is an in vitro culture system. Within this system individual cells, to a certain extent, experience an environment with similarities to true microgravity (Gao et al., 1997; Battle et al., 1999). BaF3 cells stably expressing the transfected human Epo receptor (BaF3-EpoR cells) were cultured in RPMI-1640 supplemented with 5% fetal bovine serum and either 1 unit/mL of recombinant human Epo (rhEpo) or 5 ng of recombinant mouse interleukin-3/mL (rmIL-3) in either the RCCS or in 175 cm^2 standard tissue culture flasks (control) at 37 ^oC in a humidified atmosphere of 95% air/5% CO_2, 37 ^oC for 48 hours. Cells were then harvested by centrifugation, incubated with ^{125}I-labeled rhEpo or ^{125}I-labeled rmIL-3, and the bound/free ligands were separated by centrifuging the cells through a serum cushion according to published methods (Yonekura, 1991). The radioactivity of the cell pellet was quantified by gamma scintillation spectrometry.

Starting from 0.1 nM of ^{125}I-Epo (the lowest concentration tested) upward, the specific binding of radiolabeled ligand to flask-cultured cells (■) was significantly greater than that to RCCS-cultured cells (□) ($p<0.01$) (Figure 1). At 3.2 nM (the highest concentration tested), the bound radioactivity (surface EpoR density) of RCCS-cultured cells was only 56.6% of that of the flask-cultured counterpart. The non-specific binding of radiolabeled Epo to the flask- (▲) and RCCS (△)-cultured cells were similar.

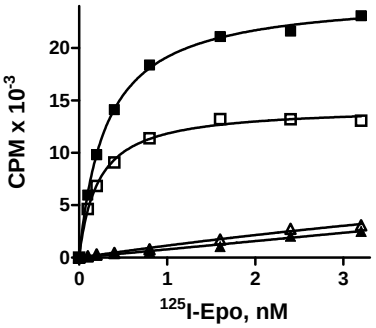

Figure 1. *The binding curves of ^{125}I-Epo to BaF3-EpoR cells grown in flasks (■) and RCCS (□). Each point shown represents the mean of triplicates ± standard errors (S.E.).*

To determine the specificity of the RCCS effect on EpoR, the abundance of IL-3 receptor was also studied. Similarly, ^{125}I-IL-3 binding to RCCS-cultured cells was significantly reduced compared to that to flask-cultured cells ($p<0.01$) (Figure 2). This result suggests that culture in the modeled (simulated) microgravity environment of the RCCS has a generalized effect on cell surface growth factor receptors.

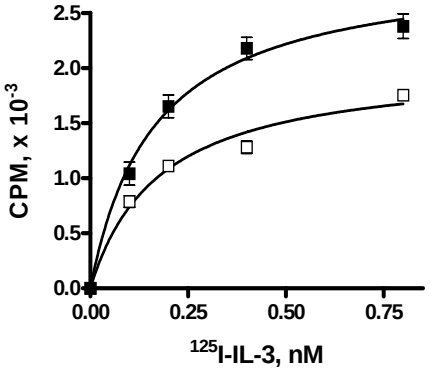

Figure 2. *The binding curves of ^{125}I-IL-3 to BaF3-EpoR cells grown in flasks (■) and RCCS (□). Each point shown represents the mean of triplicates ± S.E.*

Cell viability from both flask- and RCCS-cultured cells was assayed by trypan blue dye exclusion. Both exhibited cell viabilities of greater than 90% ($p>0.05$) (Figure 3). This result excludes the possibility that the effect of RCCS culture on cell surface receptors is due to mechanical damage to the cells or other intrinsic factors, such as toxicity.

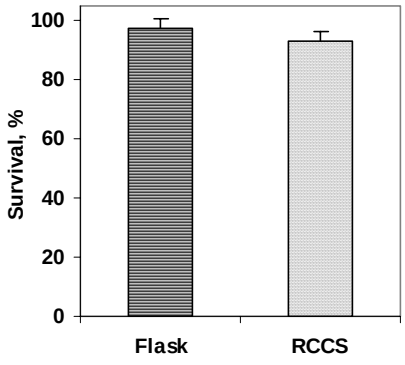

Figure 3. *Histogram of survival rates of BaF3-EpoR cells grown in RCCS and flasks. Each bar shown represents the mean of triplicates ± S.E.*

The possibility of nutrient depletion as a factor contributing to the reduction of cell surface Epo receptors was excluded by the following study. Cells were cultured in RCCS with either 1 ( ) or 2 ( ) mg/mL of glucose in the cell culture medium. No changes were observed in both specific binding (SB) and non-specific binding (NSB) between the two glucose levels (Figure 4).

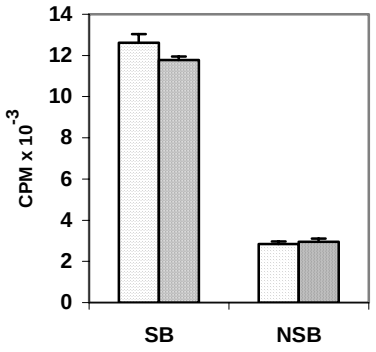

Figure 4. *The histograms of ^{125}I-Epo binding to Ba/F3-EpoR cells grown in Low ( ) and high ( ) glucose in RCCS. Each bar shown represents the mean of triplicates ± S.E.*

This is the first study showing possible microgravity effects on the EpoR and the potential involvement of the EpoR in the anemia of space flight. This study also suggests that the expression of other cell surface growth factor receptors may be affected by gravity.

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