

LEVEL OF ERYTHROCYTE INTRACELLULAR REACTIVE OXYGEN SPECIES (ROS) IS SIGNIFICANTLY HIGHER IN HEMODIALYSIS PATIENTS THAN IN HEALTHY CONTROLS

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Background and Aims

Multiple factors contribute to the anemia of chronic kidney disease (CKD). [1] While administration of EPO may correct anemia and avoid blood transfusions, high EPO dose is associated with higher mortality in HD. [2-5] To further understand mechanisms of anemia in CKD we explored levels of erythrocyte intracellular ROS and other parameters in hemodialysis patients (HD) and in healthy controls (HC).

Methods

Red blood cell (RBC) total intracellular ROS, and reticulocyte count (RETIC) were assessed in fresh Li Heparin blood samples from 55 stable HD patients (20 African-Americans; 23 women, 8 perm. Cath., age 55 yrs (23 - 80), EPO dose 368±4291 U/HD) and 7 HC (3 women, age 52 yrs (23-62) using flow cytometry with Total ROS/Superoxide Detection Kit (Enzo Life Sciences Inc., USA); and RETIC (BD Biosciences, USA), respectively, within two hours of sample collection. All clinical data were from patient charts. Total ROS/Superoxide Detection Kit uses two dyes: the non-fluorescent, cell-permeable ROS detection dye, which reacts directly with a wide range of reactive species, such as hydrogen peroxide (H₂O₂), peroxy nitrite (ONOO⁻), hydroxyl radicals (HO[•]), nitric oxide (NO), and peroxy radicals (ROO[•]), yielding a fluorescent product indicative of cellular production of different ROS/RNS types; and the superoxide detection dye that reacts specifically with superoxide (O₂⁻). Two-tailed homoscedastic T-test was used to explore associations between parameters; P<0.05 was considered significant. Data are presented as mean ± SD, * P<0.05 between HD and HC (Table 1).

Results

We found significantly higher levels of total intracellular ROS (but not superoxide) and of RETIC in HD patients compared to HC (Table 1). Hemoglobin (Hgb) levels were lower in HD. Reticulocyte count in HD correlated with EPO dose (R² = 0.18 P < 0.001), but not with ROS. The product of RETIC and hemoglobin was also significantly greater in HD than in controls (0.21 ± 0.09 vs. 0.14 ± 0.06; P=0.04) and correlated with EPO dose (r² = 0.92; P = 0.009)

Conclusions

- Increased reticulocyte count, even after controlling for anemia, in conjunction with low Hgb in hemodialysis patients indicate higher erythrocyte turnover in this population. This effect is greater among those patients receiving the highest EPO dose.
- RBC total intracellular ROS is significantly elevated in HD patients compared to healthy controls but is not significantly associated either with Hgb or reticulocyte/EPO.
- Contribution of specific ROS and nitrogen species to erythrocyte redox imbalance and relationship with anemia in HD population will require further investigation.

| | ROS, % * | RETIC, %* | Hgb, g/dL* |
|----|-------------|-----------|------------|
| HD | 49.7 ± 17.9 | 1.8 ± 0.8 | 11.6 ± 1.5 |
| HC | 32.2 ± 17.5 | 0.9 ± 0.3 | 14.4 ± 1.5 |

Table 1: Mean ± SD; * P<0.05 between HD and HC

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