



Background

CXCR4, the receptor for bone marrow stroma derived SDF-1, has recently been studied in normal hematopoiesis and hematologic malignancies.

Increased expression of this receptor by leukemic blasts has been reported by us and others to be associated with poor prognosis in acute myeloid leukemia (AML) [Konoplev et al., Cancer 2007]. Its expression was thought to be transcriptionally regulated by HIF1 α (Hypoxia-induced Factor 1 α). However, all studies have so far been carried out under unphysiological conditions, i.e. under normoxia (21% oxygen or ca. 150 mmHg). In vivo, partial oxygen pressure in tissues rarely exceeds arterial oxygen levels of ca. 80 mmHg (or 10%), and in the bone marrow is estimated as appr. 6%, as recently reported. We hypothesized that the much higher partial oxygen pressure in vitro or ex vivo has major impact on the expression of CXCR4.

Methods

Oxygen partial pressure of bone marrow aspirates was measured using commercial available gas checks. A hypoxic chamber (Figure 1) was used providing a constant oxygen content of 6%.



Figure 1: For experiments in hypoxia, a Invivo 400 from Ruskinn Technology was used.

Cell cultures were exposed for at least 1 week to hypoxia before analysis (primary samples 24 hours before analysis).

Methods

For the assessment of the impact of oxygen on leukemic cells, part of cell samples was prepared for analysis under hypoxic conditions while the other part was re-oxygenated with 21% oxygen for at least 5 minutes prior to analysis. Expression of CXCR4 in the leukemic cell lines U937, NB4, HL60 and OCI and patient samples was examined by flow cytometry, confocal microscopy and Western blotting (WB) was used.

Results

In 8 patients, partial pressure of oxygen in the bone marrow was determined as 47.5 mmHg (median) or 6.25% (Figure 2)

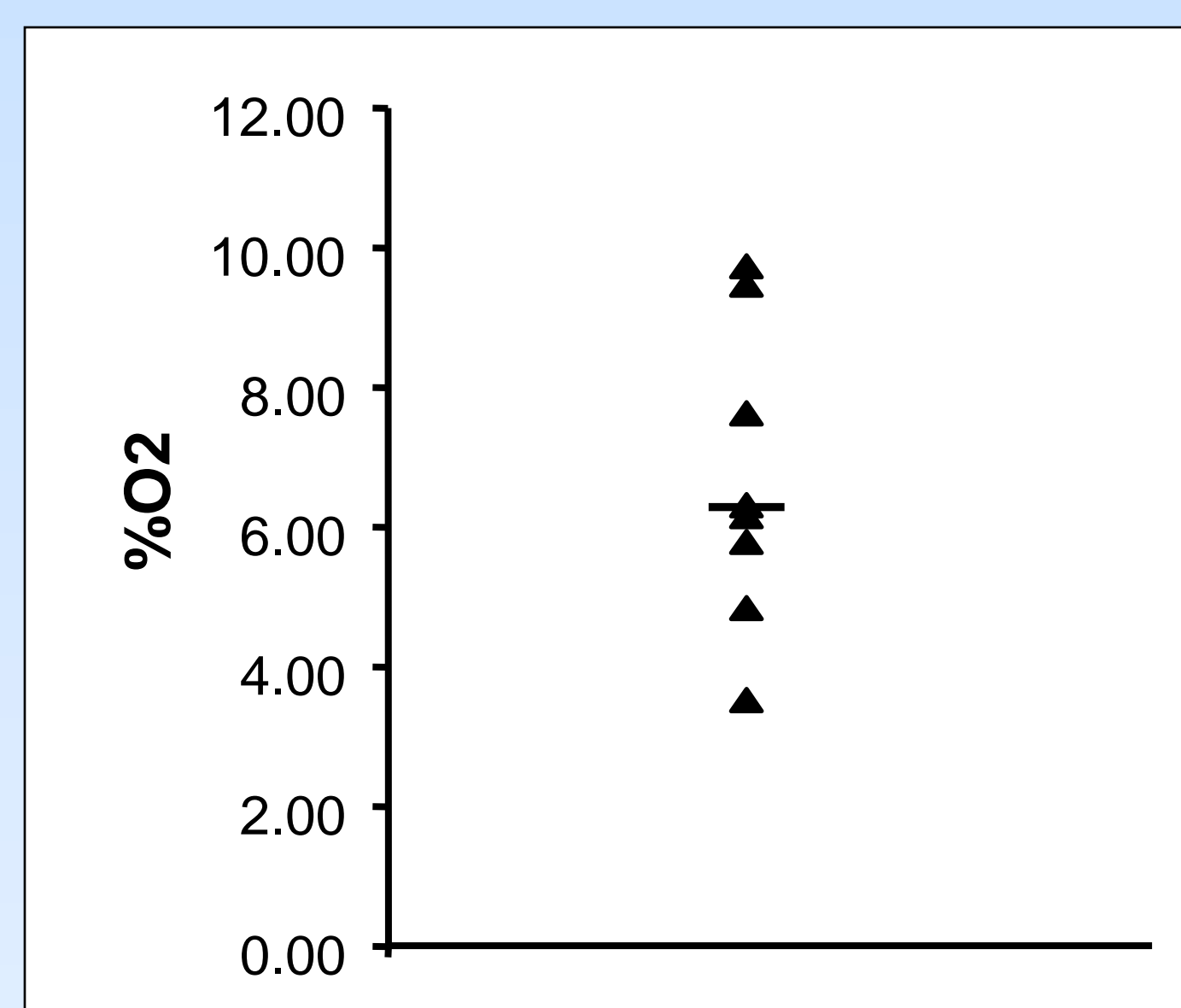


Figure 2: % Oxygen in patients without elevated blast counts. Triangle represent individual measurements, bar represents Median

This level of hypoxia significantly increases surface expression of CXCR4 in leukemic cell line U937 (MFI 484.89) as compared to parental cell lines that were kept in normoxic conditions (MFI 137.94, 3.5fold increase). However, re-oxygenation of leukemic cells resulted in a statistical significant ($p < 0.05$) degradation of CXCR4 in all examined cell lines and patient samples ($n=4$). Results are shown in Figures 3 and 4.

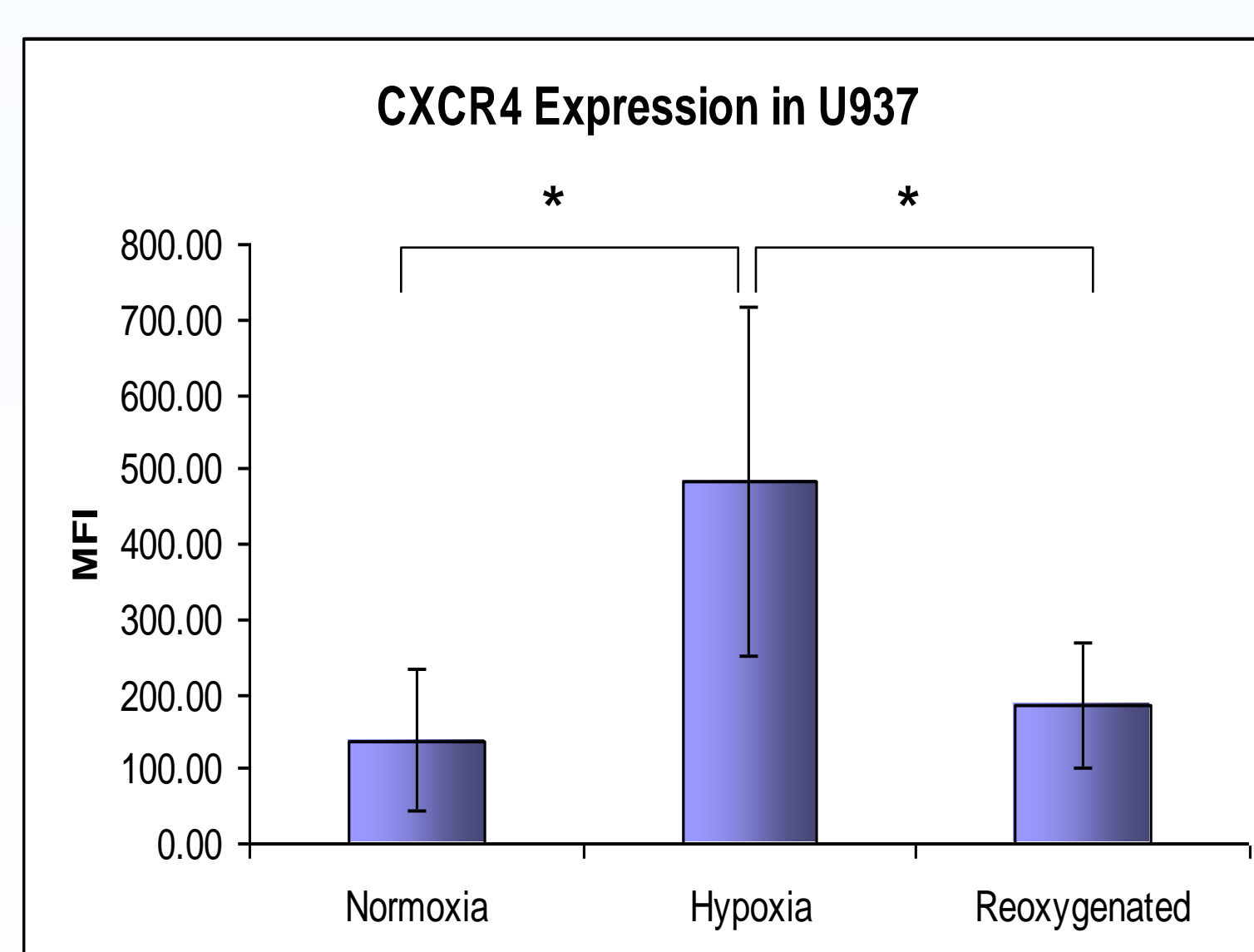


Figure 3: CXCR4 expression is significantly increased under hypoxia but lost during reoxygenation in U937 cell line. *: $p < 0.05$

Results

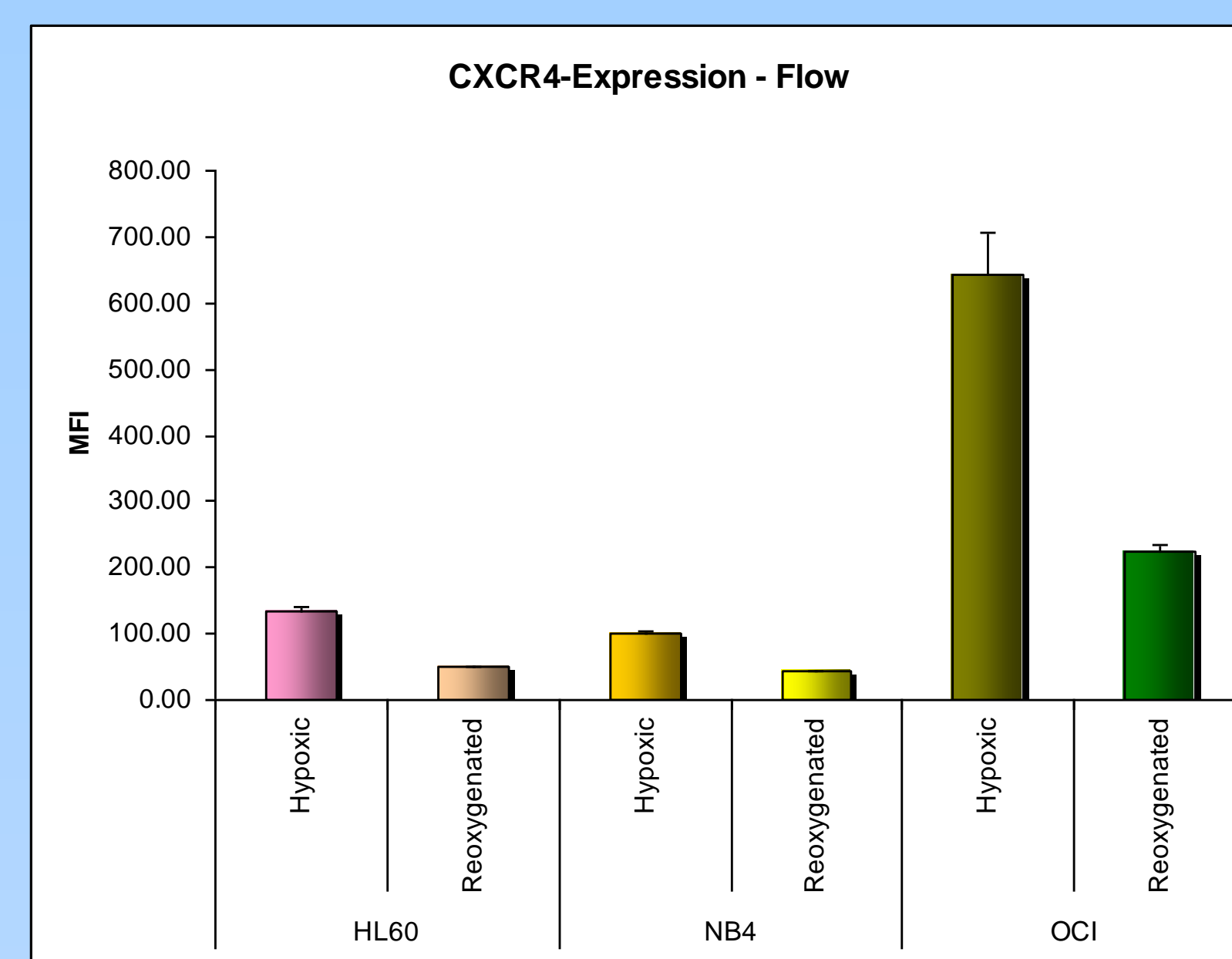


Figure 4a: CXCR4 expression is lost during reoxygenation in all examined cell lines ...

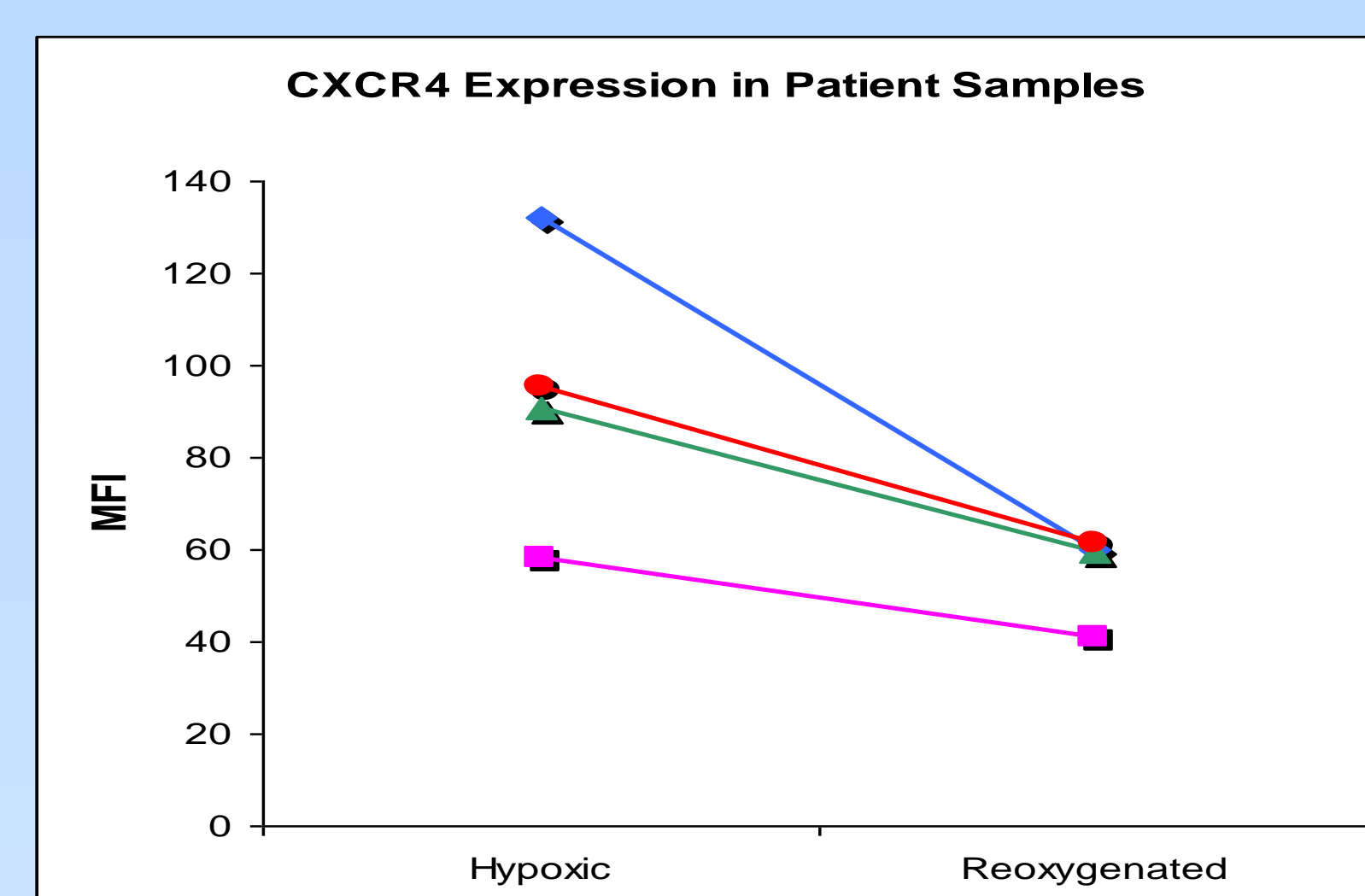


Figure 4b: ... but also in 4 primary samples from patients with AML

This loss of CXCR4 is very rapid (within 5 minutes of re-oxygenation) and was detected by flow cytometry, confocal microscopy and WB (Figure 5).

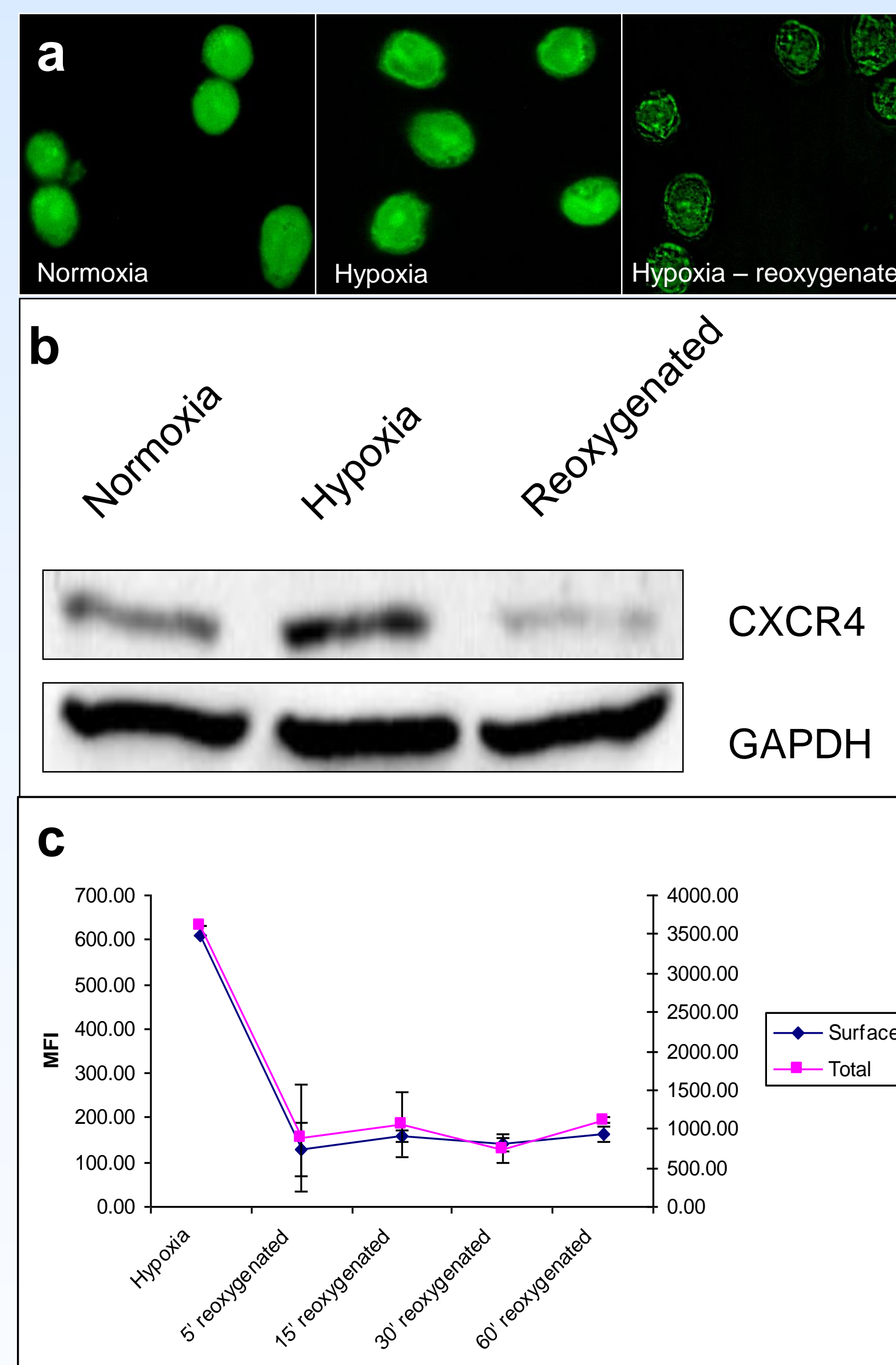


Figure 5: Confocal Microscopy (a) and Western blot (b) confirm the finding of total CXCR4 loss. Figure 5c shows a time course of flow analysis of total and surface CXCR4 in U937 cells

This phenomenon could not be prevented by proteasome inhibition (with laktacystin or MG-132), or radical oxygen and superoxide scavengers N-Acetyl-Cysteine and Tirone, and was independent of ATP (Figure 6).

Results

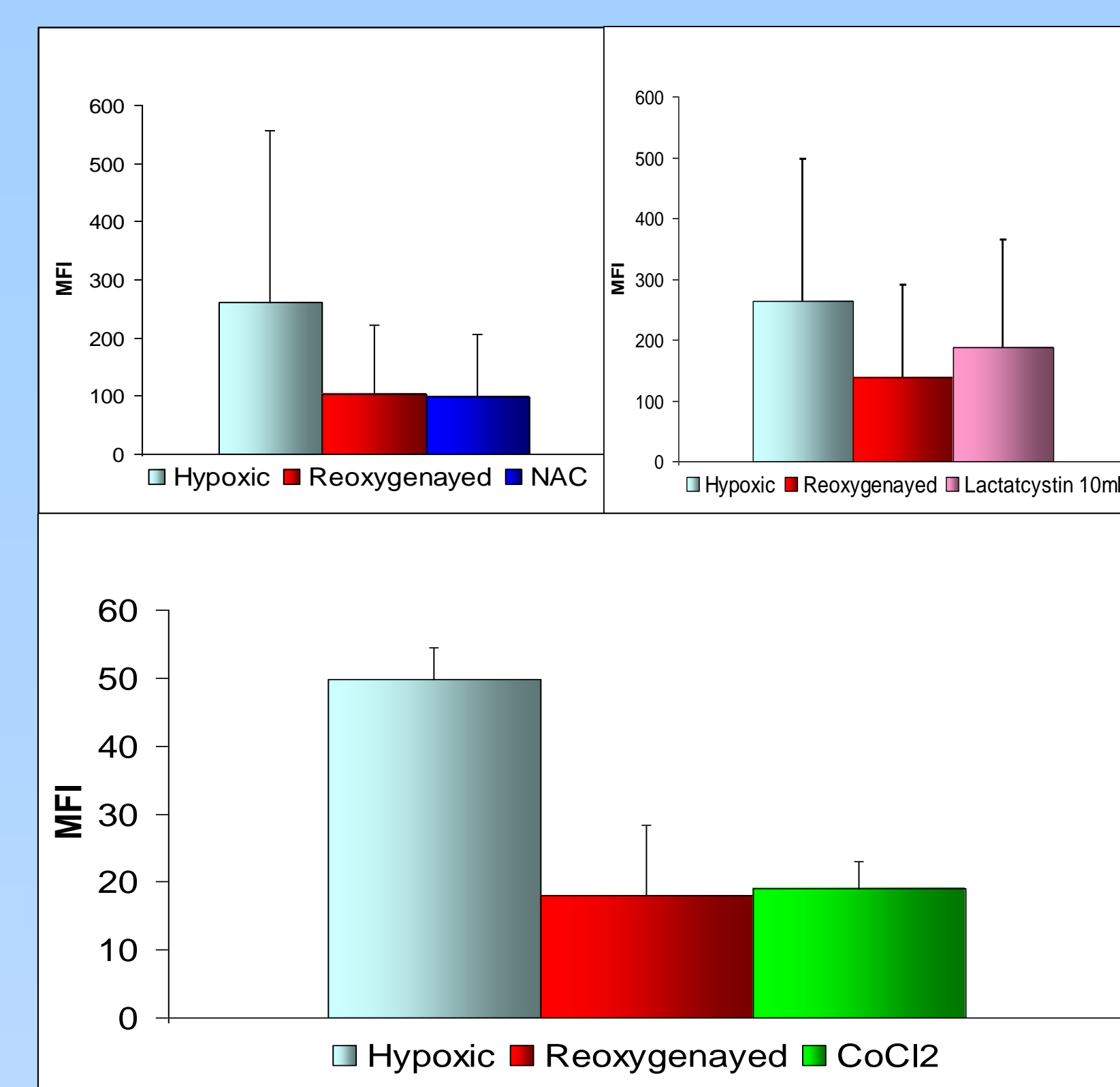


Figure 6: Loss of CXCR4 could not be prevented by NAC, Proteasome-Inhibition or CoCl2

However, as shown in Figure 7, exposure of cell lines adjusted to hypoxia to Methyl- β -cyclo-Dextrine (MbCD, resulting in a disruption of lipid rafts) led to a similar decrease in CXCR4 expression as re-oxygenation. Flow analysis for the β -subunit of cholera toxin (being part of lipid rafts) showed a similar decrease as CXCR4 during re-oxygenation.

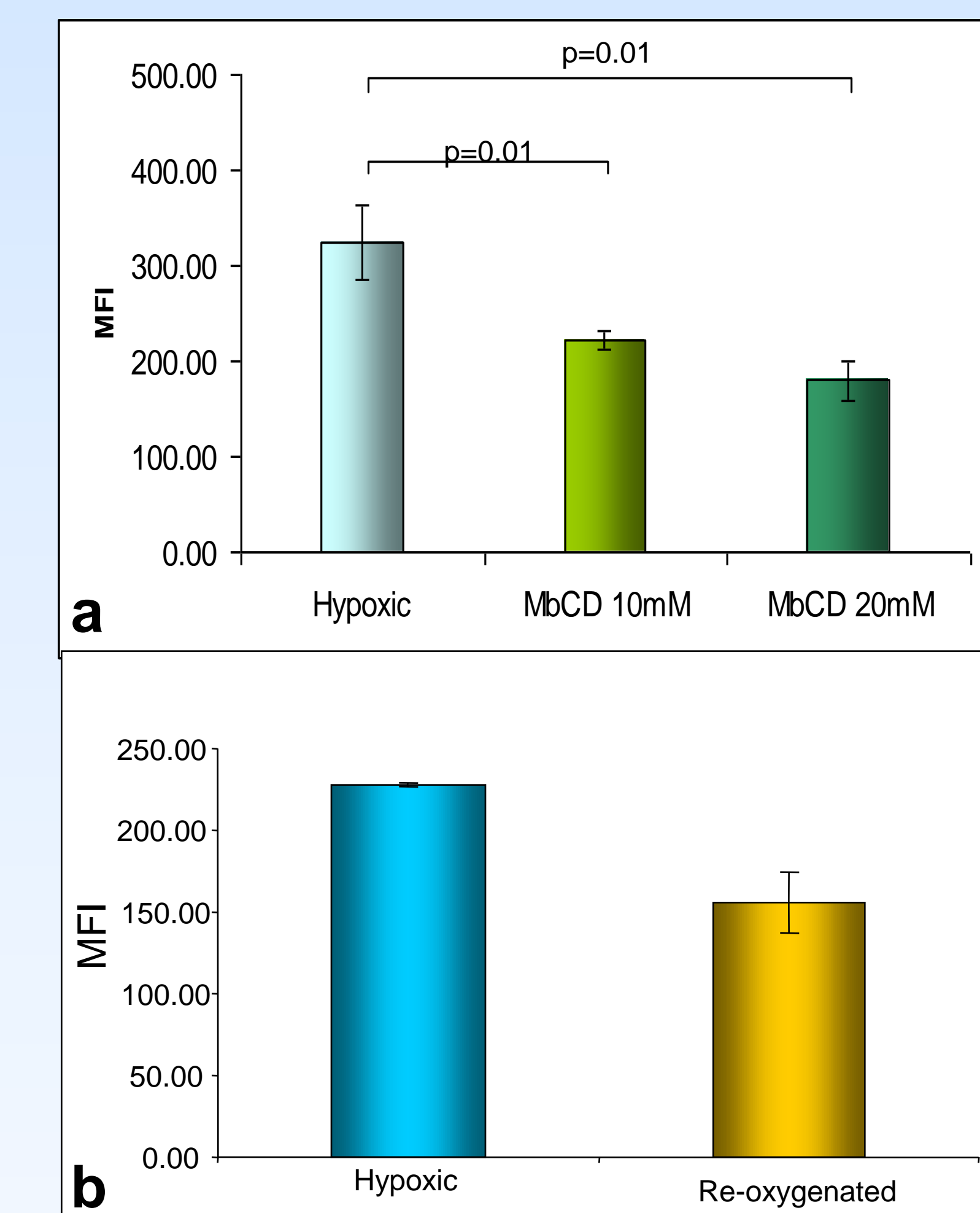


Figure 7a: Disruption of lipid rafts by MbCD leads to significant, dose-dependent decrease in the expression of CXCR4. 7b: Reoxygenation leads to a decrease in lipid rafts in the membrane of U937

Conclusion

The oxygen content of the bone marrow was determined as ~ 6%. These results suggest that increase in partial oxygen pressure in the cellular environment leads to rapid changes in the expression of CXCR4 on leukemic blasts, perhaps by changes in the formation of lipid rafts. This finding also points to the importance of studying leukemic blasts under physiologic, i.e. hypoxic conditions.