H.B.O. IMPAIRS Ca^{++} RESPONSES IN A CULTURED EPITHELIAL CELL LINE (HT29)

M. Marmo[1,2], C. Luongo[2], C. Parrillo[1], P. Pignalosa[1], V. Calderaro[1] and F. Rossi[1]

1 - *Institute of Pharmacology and Toxicology*
Faculty of Medicine and Surgery, II University of Naples, Italy.

2 - *Institute of Anaesthesiology, Analgesia, Reanimation and Intensive Care.*
Department of Hyperbaric Therapy, II University of Naples, Italy.

In order to assess the effects of hyperbaric oxygen (H.B.O.) on cellular functions, we misured the Ca^{++} signal pathway activation in HT29 colonic cells exposed to different doses of H.B.O. Hyperbaric oxygen exposures were carried out in a cylindrical steel chamber (40 cm diameter x 65 cm length-Galeazzi model - Italy). The chamber was costantly ventilated at a rate of 4 l/min to avoid carbon dioxide accumulation during pressurization. Gases were analyzed and O_2 concentration was > 99% (Taylor servomex OA 272 oxygen analyzer) and carbon dioxide below 0.2% (Med. Gas Analyzer LB-2, Model 24OM, Beckman).

Incubation of 10^6 cells/ml to 4 absolute O_2 atm for 1 hour, completely prevented the cell Ca^{++} increase produced by 500 μM carbachol, a muscarinic receptor agonist, meanwhile exposure to 2 absolute O_2 atm for 30 min blunted the Ca^{++} response to the agonist. In fact, control cells stimulated by equimolar amounts of carbachol displayed a 515±22% increase in cytosolic Ca^{++} (from 150±8 to 615±12 nM, n=4) significantly higher than that showed by cells challenged by H.B.O treatment.

In these latter, carbachol produced a 248±15% of Ca^{++} increase (from 137±6 to 475 nM, n=4, $P<0.001$). Similar behaviour was found when cells were stimulated by thapsigargin, a microsomial Ca^{++} ATPase inhibitor. Cell - staining with vital dye tripan blue revealed an elevated positive percentage (about 30-35%) of H.B.O. treated cells.

This finding shows that H.B.O. treatment impaired cell function coupled to Ca^{++} signaling pathway. It is possible that this impairment, probably caused by plasma membrane damage, as suggested by tripan blue dye exclusion test, could be the molecular basis for the beneficial effect of H.B.O. therapy.