INTERACTIONS AMONG ASCORBIC ACID, GLUCOSE AND GLUCOSE TRANSPORT INHIBITORS IN PMNs

Boscaro V., 2° anno di corso del Dottorato in Scienza del Farmaco, XVI ciclo. Durata del Dottorato in anni: 3. Sede di servizio:
Dip. Anatomia, Farmacologia e Medicina Legale – Università degli Studi di Torino.

The maintenance of antioxidant-oxidant balance is fundamental in polymorphonuclear leucocytes (PMNs) because reactive oxygen species (ROS) are amongst the main agents used by neutrophils for killing micro-organisms; on the other hand, a ROS excessive production is implicated in tissue damage in various pathological conditions [1].
Ascorbic acid (AA) plays an important role because it acts as a radical scavenger [2,3], but it is also able to affect PMNs functional activity: our previous researches show that myeloperoxidase release is inhibited by AA in a dose-dependent manner (from 30% with 0,01mM AA to 93% with 1mM AA).
Ascorbic acid can enter cells through two different mechanisms, that are temperature, energy and sodium-dependent [4,5]: a high affinity transport (Km=9mM), typically near saturation under physiological conditions; a low affinity high capacity transport (Km=2,5mM).
In many cases, transport appears to be in the form of dehydroascorbate (DHA), the oxidized form of ascorbate, followed by the rapid generation of ascorbate in target cells.
AA, DHA and glucose present structural similarities, so that DHA transport can be accomplished via the GLUT-1 glucose transporter, Na^+- independent [5].
It is known that glucose transport can be inhibited by cytochalasin B and phloretin in erythrocytes. The binding of these inhibitors to glucose transport system seems to be asymmetrical: phloretin is bound outside, cytochalasin B inside [6].
The aim of this study was to investigate the possible interaction between ascorbic acid and glucose, used at concentrations similar (1-10mM) or greater (30mM) than physiological condition, and the possible effect of glucose transport inhibition on PMNs antioxidant defences.
The experiments were performed on no stressed and stressed rats (restraint for 15 minutes – sacrifice after 0, 15 minutes, 24 hours); this stress model was chosen as it is able to induce oxidative stress, increasing ROS production. PMNs, collected from male Wistar rats, were isolated with modified Boyum method [7]. Antioxidant power was measured by FRAP assay (Ferric Reducing Antioxidant Power): this is a colorimetric method, in which absorption at 593nm, measured in a 0- to 6-minutes reaction time window at room temperature, is directly correlated to antioxidants concentration [8].
The results, expressed as means ± standard errors (at least 6 rats for each groups), are statistically analysed with the *t*-test to evaluate the significance of differences.
The obtained data underline that ascorbic acid enters PMNs in the oxidized form: as a matter of fact, treatment with 0,1mM dithiothreitol, which stabilises AA in its reduced form, causes a significant antioxidant power decrease (-51%) in pellet, with a simultaneous increase in supernatant.
Glucose is able to modulate PMNs antioxidant defences; in presence of 10µM AA, 30mM glucose causes an antioxidant power decrease both in pellet, probably due to the use of the same transporters, and in supernatant: pathological glucose concentrations induce oxidative stress and reduce cell scavenger ability.

The effects of the two glucose transport inhibitors are different: while 10µM cytochalasin B doesn't modify antioxidant defences in presence of 10µM AA, 100-200µM phloretin decreases antioxidant power both in pellet and in supernatant.
When PMNs are treated only with phloretin, FRAP values decrease only in supernatant. So we can suppose that antioxidant power reduction in pellet of PMNs treated with 10µM AA can be due to DHA transport inhibition, with simultaneous DHA accumulation in supernatant (not valuable, as DHA has not antioxidant activity); the supernatant decrease is due to phloretin-antioxidant interaction.
In pellet, the decrease obtained with 10µM AA plus 200µM phloretin is similar to that obtained with 10µM AA plus 30mM glucose, while the association of glucose and phloretin doesn't modify antioxidant power further.
Restraint influences antioxidant defences in PMNs incubated with 10µM AA and elevated glucose concentration (30mM): when animals are sacrificed immediately after stress session, antioxidant power increases both in supernatant and in pellet; yet it returns to basal value after 15 minutes in pellet. When PMNs are also incubated with phloretin, there are no stress- and time-dependent variations.
In this experimental model only phloretin modifies antioxidant power: so we can suppose the existence of extracellular binding site, but not intracellular one.
Antioxidant power decrease, observed when PMNs are treated with glucose excess, could play an important role in the explanation of PMNs reduced functional activity in pathological conditions such as diabetes.

References

1. Dale M.M, Foreman J.C. (1984) "Textbook of immunopharmacology" Blackwell Scientific Publications

2. May J.M. (1999) *The FASEB Journal* **13**(9): 995-1006

3. Halliwell B. (2001) *Mutation Research* **475**: 29-35

4. Kenneth L., Kirk, Levine M. (1995*) The American Society for Biochemistry and Molecular Biology, Inc.* **270**(21): 12584-12592

5. Patel M., McIntosh L., Bliss T., Ho D., Sapolsky R. (2001) *Brian Research* **16**(1-2): 127-135

6. Rumsay S.C., Kwan O., Xu G.W., Buran C.F., Simpson I., Levin M. (1997) *The Journal of Biological Chemistry* **272** (30): 18982-18989

7. Metcalf J.A., Gallin J.A., Nauseef W.M., Root R.K. (1986) "Laboratory manual of neutrophil function" Raven Press

8. Benzie I.F.F., Strain J.J. (1999) *Methods in Enzymology* **299**: 15-27