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Role of lipid peroxidation in ferric lactate-enhanced calcium uptake by Ehrlich carcinoma cells

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Abstract. Comparison of Ca^{2+} uptake by Ehrlich carcinoma cells in presence of ferric lactate or aluminum lactate, and formation of thiobarbituric acid-reactive substances, suggests that lipid peroxidation is associated with but not the cause of calcium overload that can lead to cell injury and death.
Key words. Iron complex; lipid peroxidation; calcium uptake enhancement.

Cell injury leading to pathological manifestations and to death is a widespread phenomenon affecting living organisms. Lipid peroxidation is considered as one of the primary key events in cell damage. However, the question has been raised as to whether lipid peroxidation is the cause or the effect of reactions leading to cytoxicity[1].

Free radical-mediated cell injury is dependent on the presence of ionic iron[2], and this cation is involved in both initiation and propagation reactions of enzymatic lipid peroxidation. In the initiation process iron converts primary reactive oxygen species ($^{\cdot}O_2^-$, H_2O_2) into $^{\cdot}OH$ or $^{\cdot}OH$-like radicals, into perferryl species, or into an $Fe^{2+}-O_2-Fe^{3+}$ species which can remove hydrogen atoms from polyunsaturated fatty acids in phospholipids. In the propagating lipid peroxidation reaction, ionic iron interacts with lipid hydroperoxides to form reinitiating radical species[3].

The principal role of intracellular Ca^{2+} homeostasis alteration during the process of cell injury is well established[4]: the subsequent excessive accumulation of Ca^{2+} provokes uncontrollable disorders in the cell metabolism leading to mitochondrial dysfunction, enzyme inhibition and denaturation of structural proteins[5]. It has been shown that ferric lactate modifies Ca^{2+} homeostasis in Ehrlich carcinoma cells[6], as well as in liver tissue[7], in vitro and in vivo and in brain tissue in vivo[8]. A similar effect has been observed in Ehrlich carcinoma cells with aluminum lactate but the mechanism involved seems to differ from the one corresponding to ferric lactate[9].

Materials and methods

In order to assess the role of iron-catalyzed lipid peroxidation in Ca^{2+} homeostasis, the uptake of Ca^{2+} by Ehrlich carcinoma cells in the presence of ferric lactate (or of aluminum lactate) was related to the extent of lipid peroxidation affecting the system. In addition, in a parallel assay the effects of albumin (which strongly inhibits the effects of ferric lactate on Ca^{2+} uptake by the cells) were compared to the evolution of lipid peroxidation. For this purpose, Ehrlich carcinoma cells (6×10^7 cells with 98% Trypan blue exclusion) were suspended in 1 ml incubation medium (140 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 12 mM $NaHCO_3$, 6 mM glucose, and adjusted to pH 7.4 with HCl) after addition of 1 μmol ferric lactate (or 1 μmol aluminum lactate) plus 1 μmol $CaCl_2$ containing 1 μCi ^{45}Ca. The cells were incubated in a water-bath with shaking at 37 °C for 30 min. 8.8 mg bovine albumin were added to a parallel series of tubes prior to incubation. A third series of tubes where radioactivity was omitted were used, after incubation, for lipid peroxidation determination. In each series, tubes with control cells (without ferric or aluminum lactates) were processed as the other tubes. After incubation the cells were washed twice with medium at 4 °C. For radioactivity counting they were dissolved in Protosol (New England Nuclear, Boston, USA) and counted in a liquid scintillation counter.

The formation of thiobarbituric acid-reactive substances by lipid peroxidation was determined as described by Gutteridge[10] with the slight modification by Fodor and Marx[11]. The thiobarbituric acid reactivity was developed by adding 0.5 ml thiobarbituric acid reagent (1% w/v thiobarbituric acid in 0.05 M NaOH), an equal volume of 25% (v/v) 2.9 M HCl, and 0.5 ml 0.15% (w/v) butylated hydroxytoluene dissolved in pure ethanol to the reaction mixture. The tubes were heated for 15 min at 100 °C and then left to cool. The resulting chromogen was extracted into 0.5 ml butan-1-ol after vortexing. After centrifugation for 15 min at 600 g the absorbance was read at 532 nm against a blank that contained all the reagents minus the cells.

Results

The table shows that the presence of albumin inhibits the effects of ferric lactate on Ca^{2+} uptake while the extent of lipid peroxidation is twice that of the control

Calcium uptake and extent of lipid peroxidation measured as thiobarbituric acid-reactive substances read at 532 nm, for 6×10^7 Ehrlich carcinoma cells incubated with ferric lactate or aluminum lactate in presence or absence of bovine albumin (mean value ± SD of 5 incubations).

	Ca^{2+} uptake nmol	Extent of lipid peroxidation (A_{532})
Cells + 1 μmol ferric lactate	15.8 ± 1.5	0.126 ± 0.014
Cells + 1 μmol ferric lactate + 8.8 mg albumin	4.1 ± 0.8	0.098 ± 0.010
Cells + 1 μmol aluminum lactate	14.4 ± 2.5	0.058 ± 0.008
Cells + 1 μmol aluminum lactate + 8.8 mg albumin	11.3 ± 3.2	0.053 ± 0.010
Cells (control)	3.8 ± 0.4	0.050 ± 0.009

cells. On the other hand, aluminum lactate increases Ca^{2+} uptake to a value similar to that produced by ferric lactate, but with a very low inhibition by albumin. Lipid peroxidation values with aluminum lactate were similar to those of control cells.

Discussion

Two important conclusions are inferred from these results: 1) there is no direct relationship between extent of lipid peroxidation and Ca^{2+} uptake, and 2) aluminum lactate, inducing an increased Ca^{2+} uptake of the same order as ferric lactate, is not inhibited by albumin and provokes no changes in lipid peroxidation.

The behavior of ferric lactate might be related to competitive binding by albumin instead of binding by glycoproteins of the cell coat, structural modifications of which, in the absence of albumin, seem to be the cause of the increased Ca^{2+} diffusion into the cell[6]. In the presence of albumin, the difference between the metal lactates could be explained as the result of the very high residual coordination capacity of ferric ion in its lactate complex form. This reactivity is reflected by its poor stability in diluted aqueous solution, where it undergoes hydrolytic polymerization leading to its precipitation[12]; precipitation is avoided by interaction with albumin, presumably by anion penetration of carboxylic groups into the iron coordination sphere[13]. Conversely, the more stable aluminum lactate does not show this interaction. In the first case, a rapid iron-albumin interaction minimizes further ferric lactate penetration into the cell coat and its binding to molecular structures which are involved in Ca^{2+} homeostasis regulation (calcium channels, ATPase, calcium-binding sites, etc.). In the case of aluminum lactate, its lower affinity for albumin permits a rapid penetration of the complex to reach those targets, with consequent effects on Ca^{2+} homeostasis. There is experimental evidence for an interaction between aluminum and calmodulin[14] and phosphodiesterase[15]. It has been suggested that aluminum induces modifications of the stereoisomerism of calmodulin and provokes a subsequent alteration of Ca^{2+}-calmodulin interaction and unrestrained Ca^{2+} influx/release into the cell[16]. In addition to this, aluminum interaction with phospholipids might be implicated in changes in rigidity and permeability at the plasma membrane level[17].

Finally, these experimental results provide new information on the questionable major role of lipid peroxidation in cell injury, and support the hypothesis that lipid peroxidation is only an associated phenomenon in the process of cell injury whose principal trigger may be the prior Ca^{2+} homeostasis disruption and overload.

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