IMPACT OF OXYGEN CONCENTRATION ON THE OXIDATIVE CYTOTOXIC RESPONSE OF MACROPHAGES

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INTRODUCTION

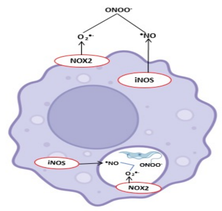
Macrophage cytotoxic response includes nitric oxide synthase- and NADPH oxidase-dependent production of nitric oxide (•NO) and superoxide ($O2^{\bullet-}$). Both enzymes use O_2 as a substrate; therefore, their activity, as well as the formation of the product of the reaction between •NO and $O2^{\bullet-}$, peroxynitrite ($ONOO^-$), could be affected by local concentrations of O_2. The objective of this research was evaluate the effect of O_2 concentration on the reactive oxygen species by macrophages and their toxicity on phatogens.

NICRIC OXIDE SINTHASE

$2O_2 + 1{,}5\ NADPH + arginine \rightarrow {}^{\bullet}NO + citrulline + 1{,}5\ NADP^+ + H^+ + 2\ H_2O$

NADPH OXIDASE

$2O_2 + NADPH \rightarrow 2\ O_2^{\bullet-} + NADP^+ + H^+$



Oxygen concetrations in differents tissues

	pO_2 %
Alveolar air	14,5
Arterial blood	13,2
Intestinal tissue	7,6
Muscle	3,8
Cell	1,3-2,5
Kidney	9,5
Liver	5,4

RESULTS

✓ Determination of •NO, $ONOO^-$ y H_2O_2 production and cytotoxic capacity in macrophage culture J774A-1 exposed to diferente oxygen concentrations.

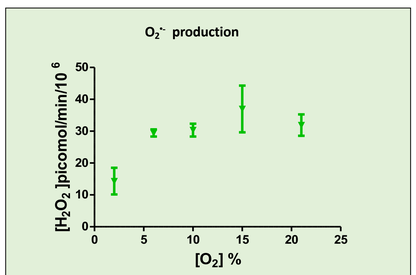


FIG 1. Oxygen peroxide formation in PMA stimulated macrophages was determined through Amplex Red technique in the presence of HRP. Fluorescence was measured in a fluorescence plate reader that controls O_2 y CO_2, during 20 minutes.

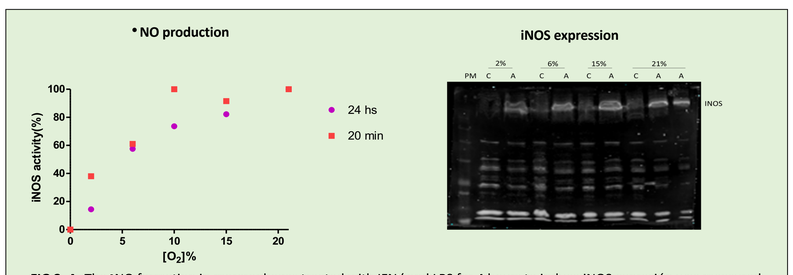


FIG 2. **A.** The •NO formation in marcrophages treated with IFNɣ and LPS for 4 hours to induce iNOS expresión was measured. After that, •NO formation speed was determined through DAF-FM oxoxidation technique, during 20 minuted in a fluorescence plate reader at different pO_2. Por otro lado, Incubations were made for 24 hours and the NO_2^- (a product of •NO oxidation) concentration was determined in the macrophage supernadant with the Griess technique. **B.** Activated macrophages were exposed to different O_2 concentrations during 7,5 hours. Starting from these cultures lysates were mades y iNOS expresión was analyzed through western blot technique.

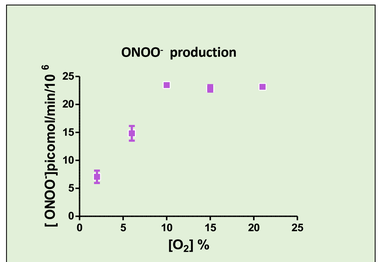


FIG 3. Macrophages were stimulated with IFNɣ, LPS and PMA for four hours. To detect ONOO- used coumarin-7-boronic acid pinacolate *(CBE)* in a fluorescence plate reader that regulates O_2 y CO_2 for 20 minutes.

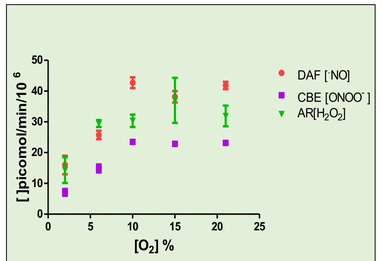


FIG 4. H_2O_2, $ONOO^-$ y •NO production

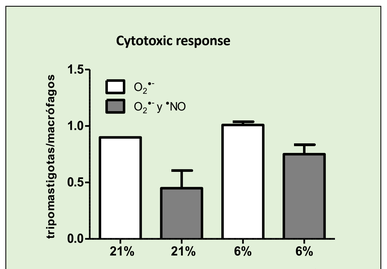


FIG 5. Macrophages (control y treated with IFNɣ/LPS) were incubated *Trypanosoma cruzi* tripomastigotes for 2 hours at 6% and 21% O_2. After that the cultures were fixated, permeabilizated and *painted* with DAPI. Macrophage/parasite relation was the measured with fluorescence microscopy in every condition.

CONCLUSIONS

✓ At 6% O2, almost a 100% of $O_2^{\bullet-}$ and 60 % •NO production is conserved and therefore so is $ONOO^-$ production.

✓ Production of •NO y ONOO- is mantained until a pO_2 of 10%, below this concentration the speed of production of these molecules is diminished.

✓ Results show that in the in the tested times there were no difference in iNOS expression,, which means that variations in •NO production is likely due to the role of O_2 as a sustrate of iNOS.

✓ Observed macrophage citotoxicity is lower at 6% tan at 21% O_2. Nevertheless, even at 6% O_2 macrophage activation to produce peroxinitrite augments its capacity to eliminate T. Cruzi, showing the relevance of this oxidant as a citotoxic agent at physiological conditions