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Effects of Lipopolysaccharides on Activity of Mitochondrial Reductase in Rat Liver Cells

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Lipopolysaccharides (LPS) are glycolipids found in abundance on the outer membrane of all gram-negative bacteria and have the ability to incite a vigorouse inflammatory response. They are among the agents that are able to induce changes in liver functions by production of nitric oxide (NO). The liver has the ability not only to clear LPS, but to respond energetically to LPS [1]. The aim of the present study is to verify the action of LPS on activity of mitochondrial reductase on rat liver cell cultures. Male rats were used, 90 days old, Wistar strain, to obtain liver cells by Berry and Friend method modified from Seglen [2]. The viability of the final parenchymal cell suspension, checked by trypan blue exclusion, was between 90% and 95%. Cells were seeded at a density of 40 x 10^4 cells/well in 24-well tissue culture plates. Cultures were grown at 37°C in an humidified atmosphere of 5% CO_2 and 95% air in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 10^4 units/ml of penicillin G, 10 mg/ml of streptomycin and 25 μg/ml of amphotericin. The activity of mitochondrial reductase has been estimated by spectrophotometrical MTT test [3]. Once happened cell adhesion, bacterial lipopolysaccharides have been added in cell culture to the final concentrations of 0-12.5-15-17.5-20-30-40-50 μg/ml for 18 hours [4]. After the 18 hours of incubation, MTT solution (5 mg/ml) was added and allowed to incubate for about 3 hours. After the medium had been discarded, the cells and dye cristals were dissolved by adding 400 μl of DMSO. The absorption was measured at 570 nm and the results were expressed as means ± S.E.M. values. The figure 1 shows as the activity of mitochondrial reductase increases in respect of the reference, catching up the maximum activity to the final concentration of 20 μg/ml. To the concentrations of 30-40 μg/ml a decrement of activity is observed, that it returned to next values to the reference for concentrations of equal LPS to 50 μg/ml.

The results demonstrated that the bacterial lipopolysaccharides, also having citotoxic action, in all the concentrations studied determine an increase of activity of mitochondrial reductase that does not come down under the reference values. The changes of mithocondrial reductase activity after incubation of parenchimal cells with LPS could be caused by the iNOS induction. In fact NOS synthesize NO, that may regulate hepatic metabolism directly by causing alterations in hepatocellular (hepatocyte plus Kupffer cell) metabolism and function or indirectly as a result of its vasodilator properties. However the potential for NO to exert cytotoxic or cytoprotective actions remains unclear [5].

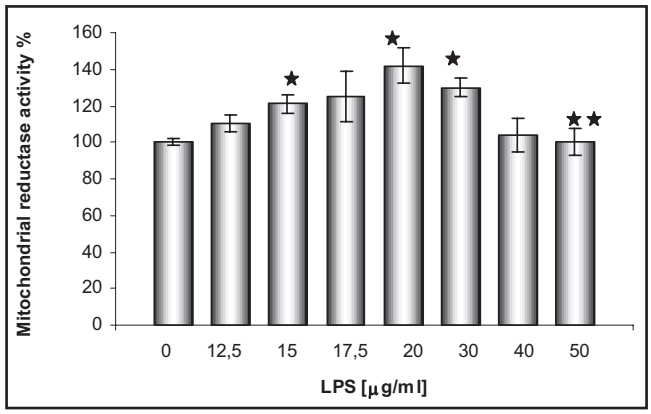

Fig. 1. Effect of bacterial lipopolysaccharides on the activity of mitochondrial reductase. The cells were exposed with indicated concentrations of LPS for 18 hours. The mitochondrial reductase activity was determined by MTT assay. Results are expressed as a percentage of the control (i.e., cell without LPS). Values are the means ± S.E.M (bars) of four indipendent experiments (P<0,05 vs control; ** P<0,05 vs 15-20-30 LPS [μg/ml])*

References

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