Association of ozone with 5-fluorouracil and cisplatin in regulation of human colon cancer cell viability: in vitro anti-inflammatory properties of ozone in colon cancer cells exposed to lipopolysaccharides

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Introduction.

Ozone therapy is an effective medical treatment for different diseases like mucositis, psoriasis, acute pain, neurovascular diseases and cancer (1,2). Emerging evidence indicates that ozone, a strong oxidant, could effectively improve organ ischemia-reperfusion, herniated disks and skin ulcers in clinical model with interesting anti-inflammatory properties through inhibition of NF-κB activation in acute and chronic disease. The paradox effect of ozone, that despite being a powerful oxidant activates the antioxidant system has been widely documented (3,4) . The aim of this study is based on the association of different ozone concentration with 5-fluorouracil and cisplatin in human colon cancer cell (HT29 cell line) in order to investigate about possible anticancer synergistic effects. Secondary endpoint of the study was based on the anti-inflammatory properties of ozone in HT29 cell line exposed to lipopolysaccharides.

Methods.

HT29 cells were incubated with ozone at different concentration ranged from 10 up to 50 μg/ml at different incubation time (2-6-12 and 24h) alone or in combination to common anticancer drugs used in clinic named cisplatin and 5- fluorouracil. Cell viability was determined looking at colon cancer mitochondrial dehydrogenase activity by means of a modified MTT method. Anti-inflammatory studies were conducted incubating HT29 with or without 20, 30 or 50 μg/ml of ozone for 30 min before exposure to lipopolysaccharides (40 ng/ml) for 12h. The culture medium without any dilution was used to assay the production of IL-6, IL-8 and IL-1β.

Results.

Ozone alone has a time and concentration dependent cytotoxicity against human colon cancer cells with an IC50 value of 30 μg/ml after 24h of incubation. Association of cisplatin of 5-fluorouracil with ozone always increase of about 15-20 % cellular cytotoxicity after 24h. Pre-incubation of ozone at 50 μg/ml decrease IL-8, IL-6 and IL-1β cellular production of about 50, 56 and 70 %, respectively, compared to cells treated only with lipopolysaccharides.

Conclusion:

Taken together, these results indicated that ozone could be useful in colon cancer management in combination to 5-fluorouracil and cisplatin with significant inhibition, under pro-inflammatory conditions, of cytokines having a central role in colon cancer cell survival and chemo-resistance (5). However, taking the several limitations of this study, further biological in vitro investigations (like biochemistry, gene expression and cell cycle studies), as well as in preclinical model, are being carried out in order to better understand the potential of ozone as possible adjuvant agent of colon cancer common therapies.

Bibliography:

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Figure1 HT29 cell viability (± SEM) performed by modified MTT method as in function of the time (from 2h up to 24h) and ozone concentration (from 10 up to 50 μg/ml) and only in function of the concentration of anticancer drugs (cisplatin tested from 5 up to 1500 μM ; 5-fluorouracil tested from 0,1 up to 100 μM) alone and in combination to ozone after 24h of incubation. *p<0.001 ;**p<0.05.

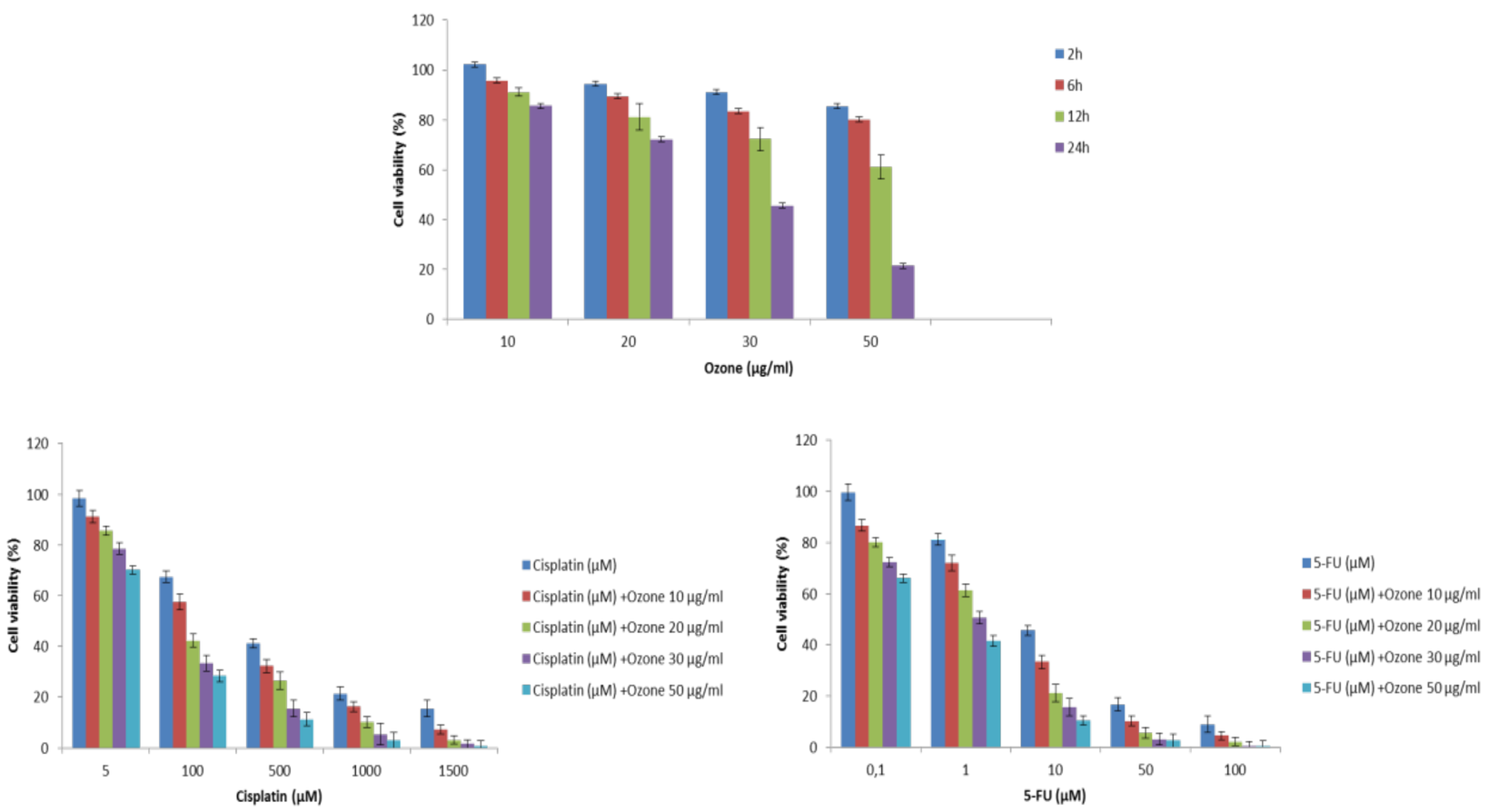

Figure2 : Anti-inflammatory properties of ozone based on affections of IL-8, IL-6 and IL-1β production on HT29 cell line (1.2 x 105 cells/well). Cells were pre- treated with or without ozone at 20, 30 and 50 μg/ml for 5h before exposure to LPS (40 ng/ml) for 12 h.