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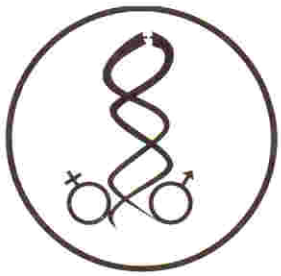

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8 Hydroxy-Guanosine, a guanosine adduct, in cultured cells incubated with 4 hydroxy Ifosfamide and during an Ifosfamide treatment in children.

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Attack of DNA by reactive oxygen species frequently results in oxydative DNA damage, notably by hydroxylation of DNA bases. These oxygen species can be generated either by endogenous reaction or by chemotherapic treatment. The hydoxylation of guanosine is one such example leading to the 8 hydroxy guanosine (8-OHdG). Damaged DNA is repaired by endonucleases or by a base-specific glycosylase. 8-OHdG induces G:C to T:A transversion during DNA replication. In order to evaluate these damages occuring in chemotherapic treatment by Ifosfamide (Ifos.) we investigated 2 different trials. One of them was the incubation of cultured cells with the active metabolite, 4 hydroxy-Ifosfamide (0.5-5µg/ml). The second trial consisted in analysis of leucocytes of patients with sarcoma diseases treated by a continuous infusion of 3g/m²/d Ifos during 5 days.

8-OHdG determination was performed using a LC method and electrochemical detection (365mv). An UV co-detection (254 nm) was used for the guanosine quantification. Blood samples were collected every day of the treatment session for leucocytes isolation. Extraction and hydrolysis of DNA were in accordance with the recommended method described by Ames *et all*. Normal range in untreated patients was comprised between 0.17 and 0.79 8-OHdG for 10^5 guanosine. Similar results were found in cells before incubation with the active compound.

Preliminary results obtained with cultured cells with 4OH Ifos. indicate an increase of this modified base in the first hour reaching 2.63 to 5.03 / 10^5 guanosines. Then a slight decrease (1.65-2.40) remains higher than normal values. The first tested patients exhibit an increase of 8-OHdG equivalent to cultured cells in the first day of the infusion. Decreases during the following days reach either a normal level at the end of the infusion or a similar level to cultured cells. These results have to be confirm on a larger sample and could interestingly be correlated with the possibilty of relaps. (Supported by a Ligue nationale grant N° 98/RS-PH/86)

METALLOTHIONEIN EXPRESSION IN BREAST CANCER: ITS POSSIBLE ROLES

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Metallothioneins (MTs) are a group of low molecular weight sulfhydryl-rich proteins involved in cell growth and multiplication. They selectively bind to heavy metals such as zinc and have metalloregulatory functions in cellular repair processes. MTs are known to have antioxidant properties and by protecting against reactive oxygen species, they are cytoprotective against free radical induced apoptosis. This would have implications on the treatment modalities of cancers, in particular chemotherapy and radiotherapy. It has been proven that expression of MT has different prognostic significance in various human tumors. In ductal breast cancer, MT overexpression appears to be predominantly associated with the more malignant, higher-grade tumors. High levels of MT expression have been associated with poorer prognosis in breast cancer. Immunohistochemical methods are able to detect MT I and MT II isoforms but are unable to distinguish between the two groups. We present data on metallothionein immunohistochemistry in breast cancer and adjacent benign breast tissues and explored the relationship of MT expression with clinicopathologic parameters such as histologic grade, tumour size, age and lymph node status. MT protein localization was performed using the primary E9 antibody (a monoclonal anti-metallothionein antibody against a conserved epitope) and visualization by standard avidin-biotin-complex technique. The relationship of (a) steroid receptor status, metallothionein expression by both immunohistochemmistry and mRNA analysis in established cell lines and breast cancer tissues and (b) tissue zinc levels evaluated by atomic absorption spectrometry and MT expression will also be presented The significance of our findings, together with recent work on metallothionein and breast cancer by other investigators will be discussed.

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EFFECTS ON SPLENIC T LYMPHOCYTES IN EHRLICH ASCITES TUMOR-BEARING MICE (EAT) INDUCED BY MAPA – ROLE OF CYTOKINES. G.Z. Justo[1], N. Durán[2], M.L.S. Queiroz[1]. [1]Dept. of Pharmacology/Hemocentre, Faculty of Medical Sciences, [2]Institute of Chemistry, Biol. Chem. Laboratory, Universidade Estadual de Campinas, CEP 13083-970, C.P. 6111, Campinas, SP, Brazil. E-mail: zenker@zaz.com.br

The immunomodulator MAPA is an extracellular aggregated polymeric form of protein magnesium ammonium phospholinoleate-palmitoleate anhydride, isolated from *Aspergillus oryzae*, having significant antitumoral properties in vivo. A remarkable consequence of tumor growth is a decline in the host immune function, partly by a diminished responsiveness of T lymphocytes. Soluble inhibitory factors derived from macrophages and the tumor itself have been described that account for this suppressive effect. We have previously suggested that one possible therapeutic effect of MAPA against EAT may be related to the number of macrophages available and the extent to which their functional activities are modulated. We now report evidence indicating that therapy with MAPA contributes to restore T cell functions. BALB/c mice bearing the EAT showed reduced spleen cell proliferation when stimulated with concanavalin A mitogen. This was accompanied by striking spleen enlargement, with a marked increase in total cell counts. Moreover, a substantial enhancement in IL-10 level, paralleled by a significant decrease in IL-2 were observed, while production of IL-4 and IFN-γ were not altered. These data suggest that the tumor induces a Th2-type response while the Th1-type response was unaffected or partly depressed. Treatment with 0.5 and 5.0 mg/Kg MAPA for 7 days reversibly restored T-cell responsiveness to the mitogen and IL-2 production. The concentration of IFN-γ was significantly increased over normal values in MAPA-treated tumor bearing mice. In addition, a 35% reduction in splenomegaly with normal number of nucleated cells were found. We suggest therefore, that MAPA up-regulates anti-cancer T cell responses by enhancing Th1 function. This Th1 trend may be related to the antitumoral effects of MAPA. Additionally, it has been suggested that IFN-γ increases macrophage-mediated cytotoxicity during cancer, favouring our hypothesis that MAPA could modulate macrophage activation towards EAT cells.
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NEW PERSPECTIVE IN IMMUNOMODULATORY THERAPY OF TUMOR INDUCED BY AN EXTRACELLULAR AGGREGATED POLYMER ISOLATED FROM *Aspergillus oryzae*. N. Durán[1], G.Z. Justo[2], M.L.S. Queiroz[2], A.N. Vieira-Matos[3], O. Rettori[3]. [1]Institute of Chemistry, Biol. Chem. Laboratory, [2]Dept. of Pharmacology/Hemocentre, Faculty of Medical Sciences, [3]CAISM-University Clinical Hospital, Universidade Estadual de Campinas, CEP 13083-970, C.P.6154, Campinas, SP, Brazil. E-mail: duran@iqm.unicamp.br

The immunomodulator MAPA, an extracellular aggregated polymeric form of protein magnesium ammonium phospholinoleate-palmitoleate anhydride, has been developed and previously shown by us to not have direct cytotoxic effects on various tumor cell lines in vitro and to possess antitumoral properties in vivo. The antitumoral advantage of MAPA was demonstrated in significant enhanced survival of treated rats and mice inoculated with spontaneous mammary carcinoma and plasmacitoma tumor cells, respectively. Treatment of Walker 256-bearing rats with MAPA resulted in 50 and 40% cures. In addition, surviving mice were resistant to rechallenge by the same tumor. In light of these findings, and the fact that progressive tumor growth is regularly accompanied by changes in the cellular constituents of the immune system, we have proposed that its antitumor effect is the result of activation of immune effector functions. In fact, MAPA was recently shown to protect mice from myelopoietic suppression caused by the Ehrlich ascites tumor (EAT) through in vitro colony assay (CFU-GM). This was accompanied by a reduction in spleen CFU-GM and splenomegaly. The compound was also found to improve survival of these mice with considerable tumor growth inhibition, suggesting a possible mechanism for regulation of granulocyte-macrophage production and expression of functional activities. In addition, our results showed significant increases in IFN-γ levels, a Th1 type cytokine, in MAPA treated EAT bearing-mice. Notably, IFN-γ is a potent macrophage activation molecule that has a positive effect in both natural resistance and adaptative immunity. It follows that the possibility of using such compound for modulating the immune response in patients with tumors offers promising new therapeutic strategies for combination chemotherapy to protect the host from hematotoxicity as well as to supplement the tumoricidal efficacy.
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