Oxygen Club of California

University of Turin

OXIDANTS AND ANTIOXIDANTS IN BIOLOGY

BOOK OF ABSTRACTS: ADDENDUM

7-10 SEPTEMBER 2005

ALBA, ITALY

OXIDANTS AND ANTIOXIDANTS IN BIOLOGY

A MEETING IN HONOR OF ANGELO AZZI

7-10 SEPTEMBER 2005
ALBA, ITALY

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OXYGEN CLUB OF CALIFORNIA
UNIVERSITY OF TURIN

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CONTENTS

Resveratrol protects against 4-Hydroxynonenal-induced apoptosis: a role for JNK, AP-1 and caspases.

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In the present study we have studied the effect of resveratrol in signal transduction mechanisms leading to apoptosis in 3T3 fibroblasts when exposed to one of the major end product of oxidized fatty acid metabolism, 4-hydroxynonenal (HNE). Mitogen-activated protein kinases (MAP kinases) and various caspases have been proposed to mediate stress-induced apoptosis in many cell types. HNE induced early activation of JNK and p38 proteins but downregulated the basal activity of ERK 1/2. We were also able to demonstrate the release of cytochrome c from mitochondria, caspase-9 and caspase-3 activation as shown by active caspase fragments and fluorometric caspase assays. Treatment of fibroblasts with specific inhibitors of MAPKs and caspases provided further mechanistic evidence for JNK, caspase-9 and caspase-3 involvement but neither p38 nor ERK 1/2 in mediating HNE-induced apoptosis. Studies undertaken by MAPK and caspase inhibitors as well as resveratrol indicated that resveratrol was as effective as JNK inhibitor in preventing JNK and caspase activation hence apoptosis. Increase in c-Jun and phospho-c-Jun and decrease in c-Fos protein levels took place within 1 h of HNE treatment which was accompanied by an increase in the DNA binding of AP-1. This effect could be overcome by pretreatment of cells with resveratrol as shown by immunoblots and gel shift assays. Overexpression of dominant negative c-Jun and JNK1

prevented HNE-induced apoptosis, which indicates a role for JNK-c-Jun/AP-1 pathway in HNE-induced apoptosis.
In light of the JNK-dependent induction of c-Jun transcription and AP-1 upregulation induced by HNE and the protective role of resveratrol, these data may show a critical potential role for JNK in the cellular response against toxic products of lipid peroxidation. In this respect resveratrol acting through MAP kinase pathways and specifically on JNK could have a role other than acting as an antioxidant.

Oncolyn inhibits telomerase activity, induce apoptosis of cancer cells, cause regression and clinical remission of solid tumors

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In general, telomerase expression is a hallmark of cancer. Telomerase activity indicates the capacity for malignant cells' unlimited proliferation and immortality. Telomerase inhibitors are attractive agents against this phenomenon.There are intense worldwide efforts, [1, 2], for this endeavor; i.e, to discover agents that selectively destroy cancer cells without harming normal tissue. Oncolyn® has been demonstrated previously to possess anti-oxidative, anti-inflammatory, anti-angiogensis, anti-metastasis, immune augmentation, dismutagenic and cytoprotective activities, as well as, promote cancer cell apoptosis.

Oncolyn was demonstrated to protect DNA against H_2O_2, silica and asbestos particles by single cell gel electrophoresis and histopathology. Effective management of malignant mesothelioma verified by CT will be included.

The present data further demonstrates the positive correlation of telomerase inhibition by Oncolyn with cancer cell apoptosis, in providing a molecular basis for Oncolyn's anti-cancer function, prevention and therapy.

Oncolyn is composed of edible plant extracts with main ingredients including flavonoids, polyphenols, phenolic acids and saponins. It protects skin from UV damage, respiratory

system from silica and asbestos, endothelium from ox LDL. It has shown anti-cancer function in nude mouse against various cancer cells implants both for prevention and therapy.
Inhibition of telomerase results in erosion of telomeres, and eventful cessation of cell proliferation and apoptosis.
Tables of telomerase inhibition with correlation of cancer cells' apoptosis and summary of clinical application of Oncolyn against various malignancies will be included.

Literature cited

1 Enzyme stopper combats cancer, Science News, May 28, 2005, 167-349
2 Efficacy of telomerase inhibitor drug discussion, American Association for Cancer Research 2005, Annual Meeting, May, Anaheim, California.

Ascorbate and nitrite react under physiologically relevant conditions to give a novel product and not NO

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Nitric oxide, an important regulator of a variety of biological functions, is synthesized by complex enzymes known as nitric oxide synthases. Recently, a novel pathway for NO production in humans that involves either direct disproportion or reduction of nitrite to NO under the acidic and/or highly reductional conditions was discovered. The latter includes ascorbic acid, which reduces nitrous acid rapidly into NO under acidic conditions. In the present work we provide direct evidence that ascorbate and nitrite react at physiological pH in 2:1 stoichiometry to yield a previously unidentified product: 3, 3'-[(hydroxyimino)bis(oxy)]bis-(ascorbic acid). The product was purified and its structure was confirmed by ^{13}C, ^{1}H-NMR, IR spectra and MS-ESI. Product spontaneously decomposes releasing NO, which was detected electrochemically, and its bioactivity confirmed with organ chamber method. Kinetics of NO release was determined by oxyhemoglobin assay. Transition metal ions (iron, copper) catalyze the release of nitric oxide. These findings are highly relevant for explaining the phenomena related to the nonenzymatic production of NO in human macro-phages and the nitrite-rich Dietary Approaches to Stop Hypertension-diet induced hypotensive effects. Furthermore, the product of ascorbate and nitrite may serve as a potential pharmacological donor of NO, which could decrease the vascular tone and increase the tissue blood flow.

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