Session 5: Antitumoral Activity of Nitric Oxide-Based Releasing Strategies: Pre-Clinical Studies Moderator: Dr. S. Perwez Hussain INVITED SPEAKERS

Utility Of Nitric Oxide And Hydrogen Sulfide-Releasing Chimeras As Anticancer Agents

Khosrow Kashfi

City University of New York Medical School, New York, USA

Background: Aspirin is chemopreventive but has significant side effects. We developed NOSH-aspirin a safer, nitric oxide and hydrogen sulfide releasing hybrid.
Aim: Here we report on NOSH-aspirin as an anti-inflammatory and its effects on human cancer cell kinetics and various cancer xenografts.
Methods: Anti-inflammatory: Carageenan rat paw edema model. Cancer cell lines: Colon, HT-29, HCT 15, SW 480; breast, MCF-7, MDA-MB-231; pancreas, MIA PaCa2, BxPC3. Normal cell lines: human mammary, HMEpC; pancreatic epithelial, ACBRI 515. Xenografts: nude mice implanted with HT-29, MDA-MB-231, MIA PaCa2 cells, were treated with NOSH-aspirin (100 mg/kg/d) or vehicle. After 3 weeks, mice were sacrificed, tumors excised, weighed, and fixed for IHC studies.
Results: NOSH-aspirin significantly reduced paw edema as function of time. NOSH-aspirin's IC_{50} in nM at 24 h for cell growth inhibition ranged from 50±2 to 250±10 in the cancer cell lines and about 400-fold higher in the normal cell lines. The cell growth inhibitory effects were due to a dose-dependent induction of apoptosis and cell cycle arrest (G0/G1), leading to reductions in cell proliferation. In xenografts, NOSH-aspirin had no effect on the weight of the mice. Tumor volume was reduced as a function of treatment time. At sacrifice, tumor mass reductions were colon: 89%, $P=0.005$; breast: 91%, $P=0.006$; pancreas: 75%, $P=0.0031$. Growth inhibition was due to reduced proliferation (decreased PCNA expression), and induction of apoptosis (increased TUNEL positive cells).
Conclusions: NOSH-aspirin is a potent anti-inflammatory, preferentially affecting cancer cells and targets parameters important in determining cellular mass.

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Antitumoral Activity Of Nitric Oxide-Releasing Compounds

Magdalena Klink, Michal Kielbik, Izabela Szulc Kielbik, Patrycja Przygodzka, Zofia Sulowska

Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland

Background: Despite significant improvements in the conventional anti-ovarian cancer therapies, tumor cell resistance to various cytostatic drugs remains a relevant problem. Therefore, the new cancer treatment strategies are being developed. Among many agents that have been studied for their potential anti-cancer activity, the most promising are the nitric oxide (NO) donors-syntethic compounds that release NO *in vivo* and/or *in vitro*.
Aim: We have evaluated the effect of NO donors on the SK-OV-3 and OVCAR-3 ovarian cancer cell lines. We assessed some of the cancer cells' specific features: the uncontrolled proliferation, overactivation of particular signaling proteins, high resistance to therapeutics and elevated expression and secretion of invasiveness/metastatic factors.
Methods: Two members of NONOates family were used: SPER/NO and DETA/NO. Cancer cell lines were cultured with different concentrations of NO donors. The cytotoxic, pro-apoptotic activity of NO donors and their impact on the phosphorylation status of STAT-3 and AKT in cells were determined. The expression of VEGF-A, MMPs and TGF-β was also evaluated.
Results: NO donors inhibited ovarian cancer cells growth making them also more susceptible to the cisplatin cytotoxic activity. Moreover, both NO donors induced apoptosis of cells and decreased activity of signaling proteins (STAT3 and AKT). Similarly, SPER/NO and DETA/NO lowered the secretion of pro-metastatic factors, responsible for cancer cells invasiveness.
Conclusions: The obtained results show that both NO donors demonstrated a wide range of action on both ovarian cancer cell lines. Therefore, they have a high potential of being a supporting compounds in the cancer therapies.

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Nitric Oxide Synthase Type III Overexpression By Gene Therapy Exerts Antitumoral Activity In Mouse Hepatocellular Carcinoma

Raúl González [a], Ángel J. De la Rosa [b,1], Santiago Romero-Brufau [c], Lydia Barrera-Pulido [d], Francisco Gallardo-Chamizo [b], Sheila Pereira [b], Luís M. Marín [d], José M. Álamo [d,1], Ángeles Rodríguez-Hernández [b], Francisco J. Padillo [d,1], Jordi Muntané [d,1]

[a] *Departament of Biochemistry and Molecular Biology, Universidad de Córdoba, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Spain*
[b] *Cirugía Oncológica, Terapia Celular y Trasplante de Órganos, Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío-Virgen Macarena/Universidad de Sevilla/CSIC, Spain*
[c] *Mayo Clinic College of Medicine, Rochester, USA*
[d] *Department of General Surgery, Hospital Universitario Virgen del Rocío-Virgen Macarena/IBiS/CSIC/Universidad de Sevilla, Spain*

Hepatocellular carcinoma develops in cirrhotic liver. The nitric oxide (NO) synthase type III (NOS-3) overexpression induces cell death in hepatoma cells. The study developed gene therapy designed to specifically overexpress NOS-3 in cultured hepatoma cells, and in tumors derived from orthotopically implanted tumor cells in fibrotic livers. Liver fibrosis was induced by CCl4 administration in mice. Hepa 1-6 cells were used for *in vitro* and *in vivo* experiments. The first generation adenovirus was designed to overexpress NOS-3 (or GFP) and luciferase cDNA under the regulation of murine alpha-fetoprotein (AFP) and Rous Sarcoma Virus (RSV) promoters, respectively. Both adenoviruses were administered through the tail vein two weeks after orthotopic tumor cell implantation. AFP-NOS-3/RSV-Luciferase increased oxidative-related DNA damage, p53, CD95/CD95L expression and caspase-8 activity in cultured Hepa 1-6 cells. The increased

[1] Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD o Ciberehd), Instituto de Salud Carlos III