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Cyclo-oxygenase inhibitors enhance hypoxic radiosensitivity in some human solid tumour cell lines through COX-2-dependent mechanisms

S. Anoopkumar-Dukie[1], E.M. McLoughlin[1], T. Conere[2], A. Allshire[1]
[1]University College Cork, Pharmacology & Therapeutics, Cork, Ireland; [2] Cork University Hospital, Medical Physics, Cork, Ireland

Radiosensitization by COX-2 selective inhibitors has been reported mainly at suprapharmacological concentrations, so COX-2 independent mechanisms cannot be ruled out. The involvement of COX-2 in hypoxic radioresistance also remains unclear. Here we tested the role of COX-2 in hypoxic radioresistance in vitro using pharmacological concentrations of COX inhibitors in parallel with RNA interference. Treated and untreated cervical cancer HeLa, breast cancer MCF-7 and melanoma MeWo cell lines were irradiated at 6.2 Gy under normoxic and hypoxic conditions (<0.1% O2 x 1h) then subcultured and allowed to proliferate. Seven days later, reduction of resazurin to resorufin was used as an index of cell growth.

COX-1 inhibitor SC560 (5μM) did not affect radiosensitivity under either normoxia or hypoxia in any of the cell lines tested. In contrast, COX-2 selective inhibitors NS398 (10μM) and SC791 (1μM) sensitized HeLa and MCF-7 cells irradiated under hypoxia but not normoxia. MeWo cells were unaffected. This radiosensitization did not correlate with COX-2 levels in the cell lines as measured immunochemically (MeWo>>HeLa>MCF7). In HeLa cells radiosensitivity was not affected either by prostaglandin E1 analogue misoprostol (5μM) or phospholipase A2 inhibitor methyl arachidonyl fluorophosphate (50 μM). RNA interference leading to 85% reduction in COX-2 expression did not affect radiosensitivity but attenuated NS398 enhancement of hypoxic radiosensitivity. This inconsistency between pharmacological and genetic targeting of COX-2 most likely reflects different effects on cell-cycle progression.

Hence COX-2 inhibitors at pharmacological concentration selectively increase radiosensitivity under hypoxia, in some but not all solid tumour cell lines. The effect is mediated through COX-2 but other mechanisms may also contribute. Therefore selective inhibition of COX-2 using these agents may provide a useful adjunct to radiation therapy.

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Combined treatment with membrane targeted apoptosis modulators and ionising radiation increases apoptotic signaling at the mitochondria

A. Rübel[1], R. Handrick[2], C. Belka[1], V. Jendrossek[2]
[1]University of Tübingen, Department of Radiation Oncology, Tübingen, Germany; [2] University of Duisburg-Essen, Institute for Cell Biology Molecular Cell Biology, Essen, Germany

Background: Radiotherapy is an integral part of current treatment concepts in the therapy of glioblastoma. The membrane active apoptosis modulators Erucylphosphocholine (ErPC) and Erucylphosphohomocholine (ErPC3) enhance the sensitivity of tumor cells towards radiation-induced apoptosis and improve eradication of clonogenic tumor cells after ionizing radiation (IR). Aim of the present study was to analyse the importance of the intrinsic cell death pathway for the induction of apoptosis after combined treatment (IR + ErPC/ErPC3) and to define the molecular details of combined action at the mitochondria.

Methods: The importance of the intrinsic pathway for the induction of apoptosis after combined treatment settings was analysed in a human glioblastoma cell line (T98G) as well as in a Jurkat T-cell lymphoma model (Bax negative, p53-negative). Functional relevance of Bcl-2-proteins for efficacy of combined action was checked in Jurkat cells with (Jurkat Bak-positive) versus without (Jurkat Bak-negative) expression of Bak as well was with (Jurkat Bcl-2) versus without (Jurkat vector) over-expression of Bcl-2. Apoptotic levels and cell cycle distribution were measured by FACS-analysis (mitochondrial membrane potential + DNA-content). Cleavage of caspases and expression patterns of different Bcl-2 family proteins after combined treatment were analysed by Western Blotting.

Results: The results obtained in the Jurkat T-cells revealed a dependence of poapoptotic action of combined treatment on the pro-apoptotic Bcl-2 protein Bak: While over-expression of Bcl-2 only delayed and decreased induction of apoptosis after combined treatment with ErPC3 and ionizing radiation, Bak-deficiency completely blocked activation of caspases and execution of apoptosis. In T98G cells the proapoptotic BH3-only proteins Bim, Noxa and Puma were up regulated by combined treatment, whereas proapoptotic Bax and Bad were activated. Similar changes were also observed in Jurkat cells. However, in Jurkat cells down-regulation of antiapoptotic Mcl-1 was a central event for cell death induction.

Conclusion: Combined efficacy of radiotherapy and ErPC/ErPC3 involves specific changes in the balance of pro- and antiapoptotic proteins at the mitochondrial level fostering apoptosis induction through the intrinsic pathway.

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Intratumoral 224Ra-loaded wires combined with chemotherapy can destroy solid malignant tumors of various histological types in mice and prolong survival

T. Cooks[1], G. Horev[1], S. Reitkopf[1], G. Marshak[2], L. Arazi[3], M. Schmidt[3], I. Kelson[3], Y. Keisari[1]
[1]Tel Aviv University, Human Microbiology, Tel Aviv, Israel; [2] Rabin Medical Center, Oncology, Petach tikva, Israel; [3] Tel Aviv University, Physics & Astronomy, Tel Aviv, Israel

Objectives: Alpha particle radiation is highly lethal for cancer cells but has so far not been used in the treatment of solid tumors due to its short range in tissue. We have developed a new approach in which tumors are treated with intratumoral 224Ra-loaded wires that continually release by recoil short-lived alpha-emitting atoms. These disperse in the tumor and deliver a lethal dose over a region measuring 3-7 millimeters in size. The proposed method was termed Diffusing Alpha-emitters Radiation Therapy (DART). The present study examines the curative effects of a combination between the 224Ra-loaded wires and anti-tumor chemotherapy, against tumors of various histological types.

Methods: Tumor cells from pancreatic (Panc02), squamous cell (SCC) (SQ2), and colon carcinomas (CT26), were injected subcutaneously into mice. Stainless steel 224Ra-loaded wire(s) (0.3 mm-diameter and 3-5 mm long) were inserted under anesthesia into tumors of 4-10 mm in diameter. Cisplatin, Gemcitabine, or 5-FU were administered concurrently. Animals were monitored for tumor development and survival. Also, an in-vitro set-up was used to assess killing of cancer cells by alpha particles.

Results: Treatment of squamous cell carcinoma with a regimen of two (5 mg/kg) i.v doses of cisplatin given concomitantly with two 224Ra-loaded wires (11.5-28.1 kBq), caused substantial growth arrest of 93%, and extended survival from 44 to 87 days. The combined treatment reduced both local tumor growth and metastatic spread to the lungs.

Pancreatic tumors in C57BL/6 mice were treated with one 224Ra-loaded wire, in combination with Gemcitabine. The combination of 224Ra wires (13-45 kBq) and Gemcitabine achieved the best local control of tumor growth. Significant reduction in tumor volume was achieved in such treated mice compared to all other treatment groups.

224Ra-loaded wires (15 kBq/wire), inhibited tumor growth of colon adenocarcinoma tumors (4-6 mm) by 45%, and even led to complete cure. Injection of 5-FU (75 mg/kg) with the 224Ra wire (17 kBq) augmented tumor destruction and growth retardation (35% compared to 224Ra and 5-FU alone, and 74% compared to no treatment).

In vitro experiments with all tumor cells exposed to 224Ra atoms revealed a dose dependent killing of the tumor cells. The combined treatment with alpha particles and chemotherapy increased apoptosis and arrested cell proliferation to larger extent than any of the components alone.

Conclusions: Diffusing Alpha-Emitting Radiation Therapy is an effective treatment to treat solid malignant tumors, and can be further potentiated in combination with chemotherapy. This combined treatment modality holds significant potential for the treatment of non-resectable human cancers.

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Radiotherapy of solid malignant human tumors in athymic mice by intratumoral 224Ra-loaded wires releasing alpha emitting atoms can achieve local tumor control

R. Etzyoni[1], T. Cooks[1], M. Tal[1], M. Schmidt[2], L. Arazi[2], I. Kelson[2], Y. Keisari[1]
[1]Tel Aviv University, Human Microbiology, Tel Aviv, Israel; [2] Tel Aviv University, Physics & Astronomy, Tel Aviv, Israel

Objectives: Alpha radiation (high LET of 100-200 keV/micron) is the most lethal form of radiation. Yet, its short range in tissue (<100 microns) has limited its use in the treatment of cancer to radiolabeled pharmaceuticals or antibodies. We developed a new method of intratumoral radiotherapy (brachytherapy) termed Diffusing Alpha-emitters Radiation Therapy (DART), based on insertion of 224Ra-loaded wires, which release by recoil short-lived alpha-emitting atoms. These atoms disperse in the tumor, leading to the formation of a high dose region through their alpha decays, and equivalent areas of tumor destruction. In the present study we examined the ability of 224Ra-loaded wires to destroy and control the development of several human-derived tumors implanted in athymic mice.

Methods: The experiments were performed on athymic mice bearing malignant human- derived tumors including prostate (PC-3), glioblastoma (GBM, U87-MG), lung SCC (NCI H520), breast (MDA-MB-231), and HNSCC (CAL-27). One or more 224Ra-loaded wires (0.3 mm-diameter and 3-5 mm long), were inserted into the tumors, and the mice were followed for tumor growth rate and survival. Tumors treated with inert wires served as controls. In vitro killing of tumor cells by alpha radiation was also assessed, on cells seeded in 96 wells plates implanted with 224Ra or exposed to alpha particle flux from a 228Th source.