We present the dosimetric validation of this technique by comparing Monte Carlo calculated dose distributions to phantom measurements. A Monte Carlo dose calculation algorithm is used since the dose delivered to bones and lungs is of central interest for TBI. For this purpose, we employ a newly developed method for performing fast Monte Carlo simulations for moving radiation sources.
Conclusions: This method could potentially replace our conventional TBI technique allowing conformal sparing of organs at risk leading to improved quality of life and higher survival rates.

913 POSTER

Recombinant Human Keratinocyte Growth Factor (rHuKGF, Palifermin) inhibits CD105 expression in mouse tongue during fractionated irradiation

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A significant reduction of radiation-induced oral mucositis by Palifermin has been demonstrated in various studies. We have shown earlier in a mouse tongue model that the protective effect of Palifermin is not only restricted to the mucosal tissue, since Palifermin also inhibited acute inflammatory changes in the blood vessels of submucosa and tongue muscle. Whether immune responses, including changes in macrophage biology are involved in the development of radiation-induced oral mucositis and underlying inflammatory reaction is not clear. Therefore, the aim of the present study was to evaluate: (1) the effect of radiation alone on the number of activated, i.e. CD105-positive macrophages in mouse tongue, and (2) the possible effect of Palifermin on these processes.
Daily fractionated irradiation, 10×3 Gy/2 weeks, was given to the snouts of the animals. One group of animals received irradiation alone. Single subcutaneous injections of Palifermin (15 mg/kg) were given to another group on day −1, relative to the first radiation fraction at day 0. Three untreated animals served as controls. Groups of 3 mice were sacrificed from day 1 to 16 and the tongues were processed for CD105 immunohistochemistry. The number of CD105-positive macrophages in the tongue tissue was counted. Additionally, the intensity of CD105 staining within the blood vessels, i.e. in fixed blood serum, was determined using an arbitrary score (0−3).
Fractionated irradiation increased the number of CD105-positive macrophages throughout the study period. Values increased from 4.5 ± 1.0 per microscopic field in controls (mean$\pm$SEM) to a maximum of 20.1 ± 4.2 at day 2 after the onset of the treatment. In clear contrast, with administration of Palifermin at day −1, no significant changes in the number of these cells were seen. Additionally, fractionated irradiation increased the intravascular level of CD105 from 0.6 ± 0.3 (arbitrary units, mean$\pm$SEM) in controls to a maximum of 2.6 ± 0.1 at day 14 after the start of the treatment. Similarly, in irradiated and Palifermin-treated animals, no significant increase in serum CD105 expression was found.
In conclusion, a single administration of Palifermin before the onset of fractionated irradiation resulted in an inhibition of the macrophage response in mouse tongue. However, additional studies are required to evaluate further the significance of these findings and their interaction with other, e.g. epithelial pathogenetic mechanism leading to oral mucositis.

914 POSTER

A novel strategy to overcome rdioresistance: selective inhibition of mitochondrial DNA polymerase gamma by vitamin K compounds

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Background: Although several molecular candidates against radioresistant mechanisms were previously proposed, few strategies have been successfully applied in clinics. Here, we demonstrate a novel strategy that selective inhibition of DNA polymerase gamma, which plays a critical role for mitochondrial DNA replication and repair, induces a strong cytotoxicity in various human cancer cells. The purposes of this study are to investigate cellular events induced by the DNA polymerase gamma inhibition by vitamin K compounds, and to explore a novel strategy against radioresistant or recurrent cancers.
Materials/Methods: Human cancer cell lines (Colorectal: HCT116 wild-type, HCT116 p53−/−, HCT15, SW620; Prostate: LNCap, PC3, DU145; Liver: HepG2, Pancreas: Panc-1, Uterus: HeLa, Breast: MCF-7, and Hematological: HL60, Raji) were used for the evaluation of cytotoxicity by vitamin K compounds (VK1, VK2, and VK3). Radioresistant clones were originally established from HCT116 cells MTT assay and colony forming assay were used for the evaluation of cytotoxicity. For the evaluation of occurrence of apoptosis, annexin-PI assay using flow cytometer was performed. Cellular superoxide and hydrogen peroxide were measured by flow cytometer analyses using dihydroethidine (HE) and 5-carboxy-dichlorodihydrofluorescein diacetate (c-DCF) staining, respectively. Mutation or heteroplasmic changes in mitochondrial DNA (mtDNA) were analysed by a direct sequencing.
Results: Cytotoxicities of VK1, VK2, and VK3 in terms of IC50 to those cancer cells were >100 μM (median, >100 μM), 25–100 μM (median, 100 μM), 5−10 μM (median, 8 μM), respectively. Interestingly, VK3 also showed a strong cytotoxicity to the radioresistant clones (IC50: 8.7 μM), while anti-cancer drugs such as doxorubicin, cisplatin, camptotecin, and taxol showed minimum effect on the radioresistant clones. Moreover, VK3 and radiotherapy showed a synergistic effect on both parental HCT116 cells and those radioresistant clones. VK3, but not VK1 or VK2, inhibited the DNA polymerase gamma activity leading to great amount of superoxide generation in both dose-dependent and time-dependent manners, while it induced minimum hydrogen peroxide generation. All vitamin K compounds did not inhibit other DNA polymerase activities. The superoxide generation caused heteroplasmies of mitochondrial DNA, and also induced apoptotic cell death, leading to cytotoxic and growth inhibitory effects by VK3.
Conclusions: VK3 could be a novel and effective strategy against various malignancies and radioresistant cells. Inhibition of the mitochondrial DNA polymerase gamma by VK3 leading to superoxide generation seemed to be a major mechanism of the cytotoxicity. Showing the synergy with radiotherapy, the DNA polymerase gamma inhibition by VK3 may pave the way to overcome radioresistance.

915 POSTER

Enhancement of glioma cell line radiosensitivity by the DNA methylating agent Temozolomide

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Background: Temozolomide (TMZ), a DNA methylating agent, is currently undergoing clinical evaluation for cancer therapy. Because TMZ has been shown to increase survival rate of patients with malignant gliomas when given combined with irradiation (IR), we investigated a possible molecular mechanism behind TMZ's radiosensitizing effect in U251 human glioma cells.
Materials and Methods: Human glioma cells (U251) exposed to [50 μM] TMZ for 1 hr followed by a change media with drug-free one were given IR with single dose of 2 Gy. At various times after IR, we investigated the Clonogenic assay, assesment of double strand DNA breakages (DSBs) and repairement, cell cycle analysis, and various types of cell death pathway after DSBs. In vivo animal study was done with 4 to 6-week-old female SCID mice.
Results: Clonogenic assay confirmed an increase in radiosensitive with dose enhancement factors of 1.32. Evaluation of γH2AX foci showed increased expression in each treated cells. Treatment with TMZ + IR did modify the time course of γH2AX foci expression in irradiated cells. At 24 hr, the number of γH2AX foci per cell expressing was significantly greater in the TMZ + IR treated cells (21.9 ± 2.14 vs. 8.43 ± 1.4 with IR alone and 7.97 ± 1.22 with TMZ alone, $P < 0.05$). However, this TMZ + IR treatment protocol did not result in a redistribution of the cells into a more radiosensitive phase of the cell cycle or in an increase in apoptosis and senescence. Mitotic catastrophe, on the other hand, was increased in TMZ + IR combination than in either single modality treatment (21.6% vs. 9% with TMZ alone and 10.3% with IR alone, $P < 0.05$). In tumor growth delay studies, the TMZ + IR combination resulted in a synergistic inhibition of tumor growth as compared with the individual modalities.
Conclusions: These results indicated that TMZ can enhance radiosensitivity and suggest that this effect may involve an increased occurrence of mitotic catastrophe following DNA damage.