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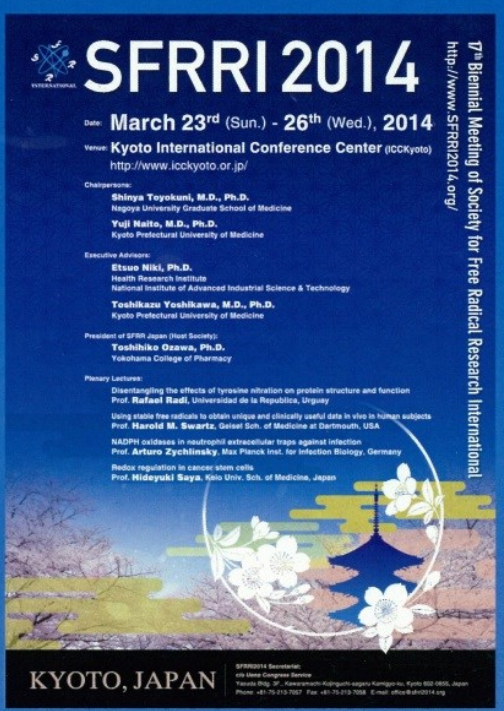

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P148

Novel photodynamic therapy with glucose conjugated chlorine for GIST

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Background: Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Except for surgical resection, no effective treatment strategies have been established. Photodynamic therapy (PDT) consists of intravenous administration of a photosensitizer, activated by a specific wavelength of light, which produces reactive oxygen species that directly kill tumor cells. We analyzed the efficacy of PDT using a newly developed photosensitizer, H2TFPC-SGlc for the GIST treatment.
Methods: Various photosensitizers were administered in vitro to GIST (GIST-T1) and fibroblast (WI-38) cells, followed by irradiation, after which cell death was compared. We additionally established xenograft mouse models with GIST-T1 tumors and examined the accumulation and antitumor effects of these photosensitizers in vivo.
Results: In vitro, the cellular uptake of H2TFPC-SGlc, and apoptosis mediated by PDT with H2TFPC-SGlc were significantly higher in GIST-T1 than in WI-38 cells. In vivo, H2TFPC-SGlc accumulation was higher in xenograft tumors of GIST-T1 cells than in the adjacent normal tissue, and tumor growth was significantly suppressed following PDT.
Conclusion: PDT with novel H2TFPC-SGlc is potentially useful for clinical applications concerning the treatment of GIST.

P149

Complexity of hemolysis and oxidative stress in patients with PNH

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal stem cell disorder, characterized by the complement-mediated intravascular hemolysis. Under oxidative stress in hematopoietic cells from patients with PNH has been reported (Exp Hematol 36, 369, 2008). Therefore, we investigated the effects of complement inhibitor, eculizumab, or a natural health food derived antioxidant, fermented papaya preparation (FPP), on the hemolysis and oxidative stress in PNH by flow cytometry measuring the levels of lactate dehydrogenase (LDH) and the reactive oxygen species (ROS), respectively. Initially, the higher ROS levels in patients than in healthy individuals were confirmed. Moreover, GPI-negative PNH type red blood cells (RBCs) showed a significant increase in ROS compared to GPI-positive normal type RBCs in patients. Eculizumab obviously improved LDH along with ROS generation. Although FPP treatment with maximum dosage showed little effect on LDH; it decreased ROS and improved the degree of fatigue. These data suggested that elevated oxidative stress in PNH was mainly due to the complement-mediated hemolysis. Eculizumab was, thus, effective in controlling the oxidative stress, in addition to the hemolysis. Since FPP showed little effect on hemolysis but had a potential to reduce the oxidative stresses, FPP could contribute to the therapeutic option for supportive therapy in PNH.

P150

Development of patient-friendly peritoneal dialysis fluid for treatment of renal failure using silica-containing redox nanoparticle

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Recently, the number of dialysis patients with chronic renal failure (CRF) has been increasing. Peritoneal dialysis (PD) offers advantages as compared to hemodialysis because PD can be carried out at home and preserves residual renal function. PD fluid with high glucose concentration, however, gives oxidative stress to peritoneum due to overproduction of reactive oxygen species (ROS) and causes encapsulating peritoneal sclerosis (EPS). In addition, the patients need 4 to 5 times exchanges of PD fluid in abdominal cavity every day. To solve these issues of PD, we have designed silica-containing redox nanoparticle (RNP) (siRNP) which is a block copolymer containing nitroxide radical as an ROS scavenger and silica nanoparticle to introduce a function as an absorbent of uremic toxins which are waste products in blood. Prepared siRNP was 40 nm in size and stable under high ionic strength environment. Administration of siRNP in abdominal cavity of the EPS model mice effectively suppresses oxidative stress against peritoneum to reduce its thickening effect. In addition, uremic toxins in blood such as urea and creatinine were significantly decreased by siRNP. These results indicate that siRNP has EPS prevention ability and removing capacity of uremic toxins such as urea and creatinine in blood effectively. Therefore, siRNP is expected as a nanomaterial for patient-friendly PD.

P151

Comparative study of the free radical scavenging activities of original and generic Edaravone determined by electron spin resonance

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Background: Edaravone, a powerful free radical scavenger, is the only drug available in the clinical practice for the treatment of cerebral infarction. Recently, many generic Edaravone injections have been commercialized. The generic injections should keep the same active ingredient, however, this rule cannot be applied to the additives. Substitution of additives has the potential effect of changing the antioxidant ability of the original Edaravone injection.
Methods: We investigated the dissimilarity between original and generic Edaravone injections focusing on their free radical scavenging activities determined by ESR.
Results: There were no significant differences in the generics in which the additives were equivalent to those of the original injection; however, the generics in which the additive L-cysteine was substituted with glycerin or citric acid showed significant reduction in their antioxidant activity toward superoxide ($p<0.01$). There were no significant differences between the original and generic Edaravone regarding the antioxidant ability toward the hydroxyl radical.
Conclusions: Our in vitro findings suggest that the antioxidant ability of generic Edaravone against the hydroxyl radical is equivalent to that of the original Edaravone and that substitution of additives in the generic Edaravone might change its antioxidant activity toward the superoxide.

P266

The effect of Fermented Papaya Preparation on radioactive exposure

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Background: Radiation (radioactive, UV) damages cells, leading to death or mutagenesis. The damage is mediated in part by reactive oxygen species (ROS). Fermented Papaya Preparation (FPP), a yeast fermentation product of Carica papaya Linn, acts as an anti-oxidant by scavenging ROS and by chelating excess cellular labile iron (LI). We studied the effects of FPP on radiation-induced damage in cultured cells and mice.
Methods: FPP (10-100 μg/ml) was added to cultured cells before or after irradiation (0-18 Gy). After 1-3 days, survival was estimated by a proliferation assay; apoptosis - by staining for phosphatidylserine exposure (with Annexin V) and propidium-iodide uptake; ROS - by staining with dichlorofluoresceine-diacetate, and LI - by calcein-AM. DNA oxidation was estimated by measuring 8-oxyguanine and DNA stability - by the "comet assay". Mice were treated with FPP (in the drinking water) before or after irradiation. Their survival and their marrow cells were analyzed.
Results: FPP significantly ($P<0.05$) ameliorated the radiation-induced increase in LI, ROS, 8-oxyguanine and DNA instability. Apoptosis was decreased and, consequently, cell survival - increased. About 60% of 14 Gy-irradiated mice who received 100 μg/ml FPP survived.
Conclusions: FPP was shown to protect cultured cells and mice against various aspects of radiation-induced damage.

P267

Oxidative stress and cell differentiation: monochloramine affects differentiation of K562 erythroleukemia cell line

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Background: Cell differentiation is an important issue not just for normal development and aging but for cancer progression and treatment. We report that a physiologically attainable oxidant, monochloramine (NH_2Cl), affects differentiation of K562 erythroleukemia cell line, which suggests that leukemic cell differentiation can be manipulated by redox control.
Methods: K562 cells (5×10^4 cells/ml) in RPMI 1640 + 10% FBS were added with NH_2Cl (60 μM), and cultured in a CO_2 incubator for the indicated times. Differentiation marker proteins (CD235, CD71, γ-globin, CD41, CD42b, CD61, CD11b and CD14, 3 d) and ERK1/2 phosphorylation (2 h) were analyzed by a flow cytometer using specific antibodies. Cell morphology was also observed by a light microscope.
Results: Erythroid markers (CD235, CD71 and γ-globin) were all increased by NH_2Cl, whereas megakaryocyte markers (CD41, CD42b and CD61) as well as myeloid markers (CD11b and CD14) did not show detectable expression. ERK phosphorylation was decreased by NH_2Cl. Interestingly, NH_2Cl induced large cells with multiple or lobulated nuclei, which was characteristic to megakaryocyte.
Conclusion: NH_2Cl increased erythroid markers in K562 cells, and the decrease in ERK phosphorylation might be involved in the mechanism. Oxidative stress may be effective in inducing leukemic cell differentiation.

P268

Variation in glucose availability induces reactive oxygen species and increased P-gp mediated multi-drug resistance to chemotherapeutics

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Background: The multidrug resistance (MDR) protein, P-glycoprotein (P-gp), reduces tumor cell sensitivity to chemotherapeutics. Moreover, within a tumor, there exists a considerable spatial and temporal gradient of glucose, rendering cells exposed to a stressful environment that the tumour needs to respond to.
Methods: After variation in glucose concentrations, reactive oxygen species (ROS) were measured by flow cytometry using the cellular and mitochondrial stress markers DCFH-DA and MitoSOX, respectively. Stress induced protein activation was determined by RT-PCR, western blotting and immunofluorescene. The effect of glucose variation on chemotherapeutic cytotoxicity was determined *via* MTT assays.
Results: Elevated and restricted glucose availability induced mitochondrial superoxide production and cytosolic stress. ROS activated the transcription factor, NF-κ β. The active p65 subunit of NF-κ β was observed to translocate into the nucleus, resulting in enhanced HIF-1α transcription. ROS also prevented PHD degradation of active HIF-1α. Interestingly, this lead to increased plasma membrane P-gp protein expression and function. Consequently, this resulted in greater drug resistance to Doxorubicin, which was reduced by the P-gp inhibitor elacridar.
Conclusion: A more aggressive MDR phenotype can result from glucose stress induced superoxide production.

P269

Nitric oxide derived from chronic inflammatory environment causes conversion of human colonic adenoma cells to adenocarcinoma cells

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We determined a mechanism of inflammation-related carcinogenesis in our established a mouse model. FPCK-1-1 cells, derived from a colonic polyp in a patient with familial adenomatous polyposis, were non-tumorigenic when 5×10^6 cells were injected subcutaneously into nude mice. However, when 1×10^5 of FPCK-1-1 cells, attached to a piece of plastic plate, were implanted in a subcutaneous space in nude mice, they were converted into adenocarcinoma cells in the chronic inflammation induced by the foreign body, a plastic plate. We found that highly proliferative fibrous stroma, formed from the plate implantation, was essential for the conversion. Further we revealed that nitric oxide (NO) derived from the fibrous stroma was the primary cause for the conversion. The conversion was inhibited by administration of NO synthase inhibitor, aminoguanidine. And FPCK-1-1 cells continuously exposed to chemically generated NO acquired tumorigenicity and resistance to anoikis (apoptosis resulting from loss of cell-substrate interactions). These results confirmed that NO was one of the causative factors for the acceleration of colon carcinogenesis, especially in the conversion from adenoma to adenocarcinoma in the chronic inflammatory environment.