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Objective: Obesity, hypertension, smoking and professional exposure to carcinogens are the recognized risk factors for clear renal cell carcinoma (cRCC). Cytosolic glutathione transferases (GST) catalyze conjugation reaction of electrophylic compounds to glutathione. Although polymorphic expression of GST enzymes confers increased risk for various cancers, the role of GST polymorphisms in susceptibility to cRCC is still controversial. We aimed to assess whether common GST polymorphisms are associated with higher risk for cRCC, independently or in conjunction with recognized risk factors as well as to identify their value in tumor progression.

Methods: A hospital-based case-control study recruited 98 patients with cRCC and 240 healthy controls. *GSTA1*, *GSTT1, GSTP1* and *GSTO1* genotypes were determined by PCR. The associations between the genotypes and cRCC risk were examined by using logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs).

Results**:** Regression analysis showed that *low activity GSTA1* (*CT/TT)* and *active GSTT1* genotypes were associated with higher, but statistically non-significant effect on risk for cRCC (OR = 1.4 CI:0.7-2.4 and OR = 1.8 CI:0.9-3.5, respectively) after adjustment for age, gender and obesity. However, when the effect of these two genotypes was analyzed in combination, patients with combined *low activity GSTA1/active GSTT1* genotype exhibited 5–fold higher risk (CI:1.1-22.9, p = 0.034) than those with *GSTA1 CC/GSTT1 null* genotype. Patients homozygous for *GSTO1 A* and *GSTP1 Ile* alleles exhibited higher, but non-significant risk compared to the patients homozygous for *GSTO1 C* and *GSTP1 Val* alleles (OR = 1.6, CI:0.7-3.8, OR = 1.5 CI:0.6-3.6, respectively). Concerning the association of GST genotypes with cRCC progression, patients with *active GSTT1* genotype or carrying *GSTO1 A* and *GSTP1 Ile* alleles had tumors of higher grade than those with *GSTT1 null* genotype or homozygous for *GSTO1 C* or *GSTP1 Val* alleles.

Conclusions: Patients with combined *low activity GSTA1* and *active GSTT1* genotypes are at higher risk of developing cRCC.

http://dx.doi.org/10.1016/j.freeradbiomed.2013.08.142

Development and in vitro proof-of-concept of interstitially targeted zinc-phthalocyanine liposomes for photodynamic therapy

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Background: Photodynamic therapy (PDT) has been used to treat numerous solid cancers. However, some cancer types respond poorly to PDT, including urothelial carcinomas, nasopharyngeal carcinomas, and extrahepatic cholangiocarcinomas. The therapeutic recalcitrance is in part due to the use of photosensitizers with suboptimal optical/photochemical properties and poor pharmacokinetics.

Objective: To circumvent these drawbacks, a second-generation photosensitizer with improved optical/photochemical properties, zinc phthalocyanine (ZnPC), was encapsulated in interstitially targeted, polyethylene glycol-coated liposomes (ITLs) intended for systemic administration. The ZnPC-ITLs were examined for reactive oxygen species (ROS) generation and oxidation capacity and validated for tumoricidal efficacy in human extrahepatic cholangiocarcinoma (Sk-Cha1) cells. ZnPC-ITL uptake as well as the mechanism and mode of PDT-induced cell death were also studied.

Methods: The ITL formulation was optimized on the basis of fluorescence spectroscopy. The extent of ROS generation, protein oxidation, and membrane oxidation were determined by the 2',7'-dichlorodihydrofluorescein assay, tryptophan oxidation assay, and calcein leakage assays using cell phantoms, respectively. PDT efficacy was evaluated by measuring mitochondrial activity and apoptosis-/necrosis-specific staining in combination with flow cytometry. The uptake of fluorescently labeled ITLs was assayed by confocal microscopy, flow cytometry, and fluorescence spectroscopy.

Results: ZnPC-ITLs exhibited maximum ROS-generating and oxidation potential at a ZnPC:lipid molar ratio of 0.003. PDT of Sk-Cha1 cells incubated with ZnPC-ITLs induced cell death in a lipid concentration-dependent manner. The mode of PDT-induced cell death comprised both apoptosis and necrosis, with necrotic cell death predominating. Post-PDT cell death was attributable to pre-PDT ZnPC-ITL uptake by cancer cells, which was more efficient at smaller ITL diameters and a more positive surface charge.

Conclusions: ZnPC-ITLs constitute a nanoparticulate photosensitizer delivery system capable of inducing apoptosis and necrosis in cultured extrahepatic cholangiocarcinoma cells by PDT-mediated oxidative processes. Animal studies are underway to provide in vivo proof-of-concept regarding the utility of this formulation in PDT.

http://dx.doi.org/10.1016/j.freeradbiomed.2013.08.143

Radical mediated degradation of cereal β-glucan

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Cereal β-glucan possesses well-established health benefits, namely the improvement of glucose metabolism and the decrease in the level of cholesterol in the case of hypercholesterolemia. These functionalities have often been related to the ability of β-glucan in forming highly viscous solutions, a feature which is directly controlled by the concentration and the molecular weight of the polysaccharide. However, β-glucan in solution undergoes a non-enzymatic degradation in presence of iron(II), which could alter its viscosity–related health benefits. This degradation process has been attributed to a hydroxyl radical-mediated oxidative cleavage, however no direct link between the formation of hydroxyl radical and β-glucan degradation has been reported, moreover the mechanism of the oxidative cleavage is not known. In the present study, we demonstrated that the presence iron(II) with a reducing agent (ascorbic acid) in β-glucan solutions causes the formation of a large amount of hydroxyl radicals, which further degrade the polysaccharide. The mere presence of iron(II) in β-glucan solutions also promoted the formation of hydroxyl radicals, hence β-glucan degradation, although to a significantly lower extent. Moreover, the radical mediated degradation of β-glucan was fully inhibited by catalase and slowed down by superoxide dismutase, which indicates that superoxide and hydrogen peroxide are intermediate species occurring during the generation of hydroxyl radical responsible β-glucan degradation. Additionally, the characterization of β-glucan oxidation products obtained

after treatment with hydroxyl radical generating system (H_2O_2/Fe^{2+}) shows that the hydroxyl radical mediated degradation is accompanied by the formation of peroxyl radicals and new oxidized functional groups, as detected by ESR and NMR, respectively. More importantly, the results indicate that the cleavage is initiated by the formation of a carbon centered radical at the anomeric carbon (C1). Thus it demonstrates that the hydroxyl radical cause the degradation of beta-glucan while changing its structural properties with the introduction of new functional group.

http://dx.doi.org/10.1016/j.freeradbiomed.2013.08.144

Influence of dietary restriction on astrocytic response in the rat brain following traumatic brain injury

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Traumatic brain injury (TBI) is a widespread cause of death and adult disability. Besides primary loss of neurons, secondary injury, which is the following event, leads to further neuronal damage and loss. One of the hallmarks of the secondary injury is microglial activation resulting in increased cytokine production and subsequent astrocytic activation. When activated, astrocytes create physical and chemical barrier around the damaged tissue which prevents axonal regrowth. This research was aimed to examine whether dietary restriction (DR) affects astrocytic response and thus modulates processes of recovery after cortical injury. Astrocytic response was followed by measuring expression of delta isoform of glial fibrillary acidic protein (GFAPσ), galectin-1 and neurocan; molecules that are mainly expressed by astrocytes and have important roles in neuronal regeneration after TBI. In this study we used male Wistar rats (3 months old) which were subjected to DR (3 months on 50% of the daily food intake) prior to stab injury in the somatosensory cortex. Tissue was collected in several time points (2, 7, 14 and 28th day post-injury) and using Western blot and immunohistochemical analyses (IHC), the level and localization of GFAPσ, galectin-1 and neurocan were examined. Our results have shown that levels of GFAPσ, galectin-1 and neurocan were downregulated in DR animals compared to animals that had unlimited approach to the food. These effects were most notably 2 and 7 days after the injury. Additionally, IHC revealed cell type specific pattern of expression of those molecules in the injured area, which was also modulated by DR. Notwithstanding that our study demonstrated that DR prior to an acute brain injury affects astrocytic response, the exact mechanism remains unclear. Revealing it might be of interest for potential therapeutic application.

http://dx.doi.org/10.1016/j.freeradbiomed.2013.08.145

Application of fluorous chemistry for the identification of carbonylation sites from oxidised proteins

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Protein carbonylation is an irreversible oxidative modification, affecting both peptide backbone and amino acid side chains. It triggers structural and functional changes to proteins often leading to their proteasomal degradation. Protein carbonylation is a well-known hallmark of oxidative stress-related diseases such as *diabetes mellitus* and Alzheimer's disease. Therefore understanding mechanism of action and characterisation of carbonylated proteins might facilitate treatment of such diseases in the future. Analytically, carbonylation is one of the most challenging post-translational modifications to study. This is due to the high reactivity, low stoichiometry and reduced ionisation efficiency of carbonylated species. To overcome these limitations enrichment of carbonylated peptides is crucial prior to mass spectrometry-based identification of carbonylation sites. We have chosen a strategy based on fluorous chemistry, known as a highly selective and efficient method for enrichment and identification of various post-translational modifications. We have adapted and optimized it for analysis of carbonylome, carbonyl content of proteins. We have synthesized a fluorous tag containing carbonyl-reactive hydrazide moiety. Utilising this tag we are able to label and enrich aldehyde-containing model peptides by fluorous solid phase extraction with a recovery of above 60%. Application of our strategy to analysis of carbonylated BSA provided information about 51 carbonylation sites, 30% of which has been reported previously. Final validation was analysis of rat liver mitochondrial proteome under oxidative stress. We have identified numerous proteins carrying at least one carbonylation site. Amongst these, proteins involved in oxidative stress-response, i.e. Proteasome activator complex subunit 1 and mitochondrial respiratory complex, i.e. ATP synthase subunit beta. Interestingly, carbonylation sites were also associated with proteins of mitochondrial DNA replication machinery. We believe that our methodology will bring insights into the role of oxidative stress in cellular homeostasis and will facilitate treatment of oxidative stress-related diseases in the future.

http://dx.doi.org/10.1016/j.freeradbiomed.2013.08.146

The mechanisms behind the inhibition of cytokine-induced inflammatory response by Cyanidin 3-Glucoside and Resveratrol in human intestinal cells: comparison with 5-ASA

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Polyphenols are naturally occurring compounds widely spread in human diet. The advantage of polyphenols in the prevention and treatment of chronic inflammatory diseases has been described by several studies; however the precise mechanisms and targets involved in cellular signaling are still not fully understood. The aim of this study was to assess the protection afforded by two dietary polyphenols, Cyanidin 3-Glucoside (C3G), a typical anthocyanin that belongs to the flavonoid group of polyphenols, and Resveratrol, a non-flavonoid polyphenol, against cytokine-induced inflammatory response in the human intestinal HT-29 cell line, in comparison with 5-aminosalicylic acid (5-ASA), a well-known anti-inflammatory drug, commonly used in inflammatory bowel disease. For this purpose, some key inflammatory mediators and pro-inflammatory enzymes were evaluated. HT-29 cells were pretreated with 25 μM C3G or 25 μM Resveratrol