Supported by HL098032

doi:10.1016/j.freeradbiomed.2012.10.490

449

Bioreductively-Activated Prodrugs of Nitric Oxide (NO)

Harinath Chakrapani[1] *and Kavita Sharma*[1]
[1]*Indian Institute of Science Education and Research, India*

Approximately one-third of human tumours contain areas with limited oxygen tension (hypoxia) in comparison with normal cells. As oxygen is a potent radiosensitizer, hypoxic tumours are often less responsive to radiation treatment. Due to diminished and chaotic blood supply, hypoxic areas are poorly accessible to cancer drugs and hence chemotherpeutics also have diminished efficacy in shrinking hypoxic tumours. Furthermore, therapy selects for cells that have a highly invasive and malignant phenotype with increased angiogenesis further complicating treatment of hypoxic tumours. Together, these effects lead to higher levels of treatment failures in tumours with significant areas of hypoxia and hence, developing new strategies targeting hypoxic cancer cells is necessary. Nitric oxide (NO) is a potent chemical radiosensitizer and has been shown to synergize with radiation to promote apoptosis in hypoxic cells. Tumoristatic activity of NO has been demonstrated by both in vitro and in vivo studies. Furthermore, NO is an inhibitor of hypoxia inducible factor (HIF-1), which enhances blood vessel growth and increases glycolytic enzymes in hypoxic cells. Finally, due to its diffusible nature, nitric oxide can in small amounts penetrate cells leading to enhancement in cytotoxic effects. Thus, NO is a potential drug candidate for targeting hypoxic tumours. Here, we report our results pertinent to the design, synthesis and evaluation of prodrugs of nitric oxide that can be used to selectively target cancer cells which over-express bioreductive enzymes such as DT-diaphorase. Our initial evaluation shows that these compounds may have therapeutic potential against cancers that over-express this enzyme.

doi:10.1016/j.freeradbiomed.2012.10.491

450

Nitric Oxide Metabolism Plays a Crucial Role in Visual Pattern Memory in *Drosophila*

Qinlong Hou[1] *and* ***Chang Chen***[1]
[1]*Institute of Biophysics, Chinese Academy of Sciences, China*

Nitric oxide (NO) plays an important role in learning and memory which is essential for animals to adapt to the external environment. However, little is known about the role of NO metabolism in this process. S-nitrosoglutathione reductase (GSNOR) is a key protein in the control of NO metabolism and protein S-nitrosation. To study the relationship between NO metabolism and learning and memory, the expression of gene *fdh* which is homolog to mammalian *GSNOR*, was modulated by the Gal4/UAS system in *Drosophila*. The over-expression of the *fdh* in the central nervous system significantly increased GSNOR activity and induced visual pattern memory defects of *Drosophila*. The role of *fdh* in learning and memory was independent of development and was neuron-specific: over-expression of the *fdh* in the fan-shaped body induced the memory defect, while over-expression in the mushroom body not. The visual pattern memory defect could be rescued by co-expression with exogenous cGMP-dependent protein kinase (PKG). Moreover, *fdh* over-expression resulted in denitrosation of multiple proteins functionally enriched in vesicle-mediated transport, which is important for learning and memory. These results showed that regulation of NO metabolism plays an important role in learning and memory, and the mechanism may involve both NO-cGMP-PKG signaling pathway and S-nitrosation modification.

Acknowledgment: This work was supported by the '973 Program' (2006CB911001, 2005CB522804).

doi:10.1016/j.freeradbiomed.2012.10.492

451

(-)-Epicatechin Increases NO Bioavailability and Nrf2-Dependent Response in the Vessel Wall in Vivo

Miriam M Cortese-Krott[1], *Thomas Krenz*[1], *Ana Rodriguez-Mateos*[2], *Friederike Oberle*[1], *Kim Weber*[1], *Sivatharsini Thasian-Sivarajah*[1], *Malte Kelm*[1], *and Christian Heiss*[1]
[1]*Heinrich Heine University of Düsseldorf, Germany* [2]*University of Reading, UK*

The oral intake of cocoa flavanols increases circulating nitric oxide (NO) metabolites and improves endothelial function in humans. This effect remains even after (-)-epicatechin, the major flavanol monomer present in cocoa, and its structurally related metabolites have been cleared from plasma. In vitro experiments suggest that flavanols contribute to sustain the redox state of cells by inducing the expression of Nrf2-dependent phase II antioxidant genes. We hypothesized that (-)-epicatechin chronically improves vascular function by increasing vascular NO bioavailability and maintaining the organ redox state in vivo via activation of Nrf2-dependent antioxidant protective genes. Oral administration of (-)-epicatechin at 1-10 mg/kg body weight over 5 days dose-dependently increased the circulating levels of epicatechin metabolites along with improved vascular response to reactive hyperemia in C57BL/6 mice as assessed by laser Doppler perfusion imaging (LDPI). These effects were abolished when the mice were pretreated with the specific NO synthase (NOS) inhibitor L-NNA, indicating that effects of (-)-epicatechin were NOS-dependent. In aortas we found increased eNOS phopsphorylation and cGMP levels, demonstrating enhanced NOS activity and NO bioavailability. Interestingly, we observed a parallel increase in the expression of Nrf2-dependent phase II genes, including glutamate cytseine ligase (Gclc) in the aorta, and GSH levels in the heart and lung of the mice. To examine the role of Nrf2, human endothelial cells were incubated with (-)-epicatechin. In culture medium (-)-epicatechin was stable ($t_{1/2}$ = 6-8 hours), did not induce H_2O_2 production, and epicatechin/metabolites were detected within endothelial cells. This induced the translocation of Nrf2 into the nucleus of the cells and the binding to the ARE. This was followed by increased expression of GCL (mRNA and protein), as well as eNOS expression and phosphorylation. Taken together, our findings suggest that Nrf2 plays a central role in mediating the protective effects of cocoa flavanols on vascular function.

doi:10.1016/j.freeradbiomed.2012.10.493

452

Nitric Oxide can Modulate Cell Death Induced by Detachment

Paulo Eduardo da Costa[1], *Roberta Eller Borges*[1], *Wagner Luiz Baptista*[1], *and Hugo Pequeno Monteiro*[1]
[1]*UNIFESP, Brazil*

Metastasis involves tumor cell migration from the primary site and