Novel Carbon-Based Particle Antioxidants in Reversible Hyperglycemic MCA Occlusion

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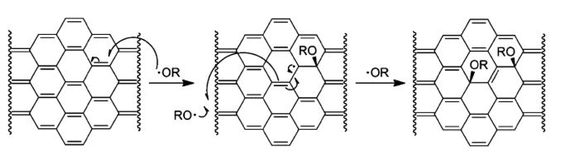
Introduction

We have demonstrated that nanoscale polyethylene glycol (PEG) conjugated oxidized carbon materials, which we refer to as hydrophilic carbon clusters (PEG-HCCs), have antioxidant characteristics favorable for their use in ischemia. These materials are rich in conjugated carbon double bond domains, and unlike conventional antioxidants, are able to quench rather than transfer oxidative radicals thus not requiring regeneration (Marcano et al, JNT in press).



Radical annihilation mechanism at a graphitic domain of the carbon particle. Two additions of RO• result in the loss of two CC π-bonds and the formation of two new CO σ-bonds and one new CC π-bond, without any radical species remaining. Only one regioisomer is shown, though many others can form by resonance of the conjugated radical

These carbon particles have shown no acute or 6 week toxicity in rodents, excellent uptake into endothelial cells and protection against TBI-induced neurovascular dysfunction (Bitner et al, ACS Nano, 2012), but were not designed for blood-brain barrier transport.

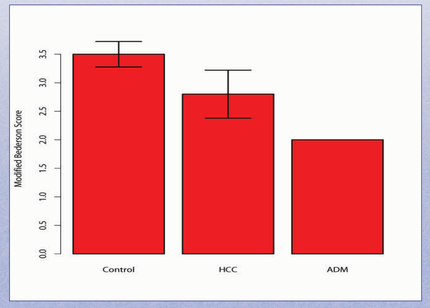
We were able to synthesize PEG-HCCs bound to adamantane (ADM), a well characterized moiety that increases lipophilicity. We tested these agents in a severe example of oxidative stress, reversible middle cerebral artery occlusion (MCAO/R) in acutely hyperglycemic rats (Martini and Kent, JCBFM 2006).

Methods

- Male, Long Evans rats received an IP injection of streptozotocin or vehicle 2 days prior to 90 minutes MCAO/R using the suture technique.
- Physiological and post-op monitoring was performed.
- PEG-HCCs or ADM-PEG-HCCs were injected IV at suture removal.
- The endpoints investigated thus far are: behavior; nitric oxide (NO) expression, reflected in the ratio of perivascular diaminofluorescein diacetate intensity in penumbra to contralateral region after 5 min. circulation (Fabian and Kent AJP 2008); and presence of the PEG-HCCs detected by reaction product from an anti-PEG antibody to the tightly bound PEG moiety.

Results

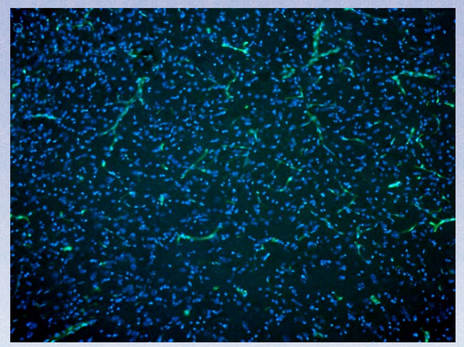
- Nanomolecular carbon particles improve behavioral deficits in MCAO/R with hyperglycemia:



HCC = Carbon particles conjugated to polyethylene glycol (PEG). ADM = Carbon nanoparticles conjugated to PEG and adamantane.

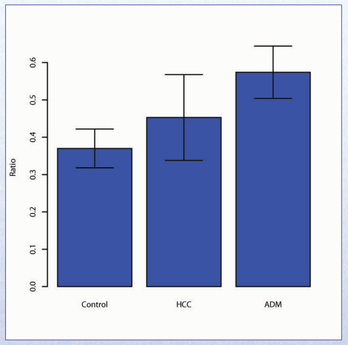
Results

- Diaminofluorescein reaction product indicates the production of NO in vascular tissue:



Green fluorescence in cerebral vasculature indicates production of NO. Blue fluorescence = DAPI nuclear counterstain.

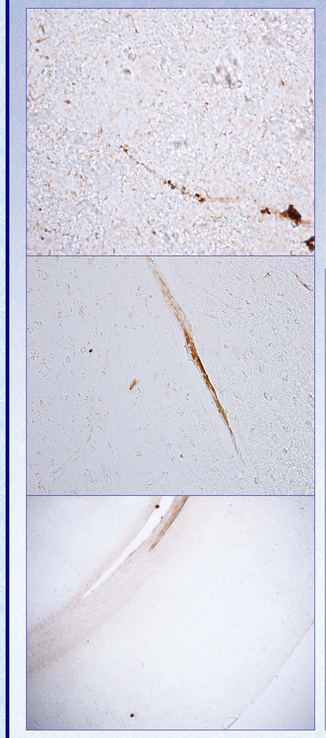
- Vascular nitric oxide in the infarct penumbra is increased by nanomolecular carbon particles:



HCC = nanomolecular carbon particles conjugated to PEG. AMD = carbon particles conjugated to PEG and adamantane. Ratio = the intensity of diaminofluoroscine reaction product in the infarct penumbra perivascular tissue to the intensity in the contralateral perivascular tissue. This indicates a reduction in vascular nitric oxide in penumbral vasculature.

Results

- Adamantane conjugated carbon particles penetrate into cerebral parenchyma uniformly:



Photomicrographs of cerebral tissue stained for the presence of PEG with an immunohistochemical technique. Brown reaction product indicates the location of PEG conjugated HCCs. The top photo is from an animal injected with adamantane conjugated PEG-HCCs, the middle from PEG-HCCs without adamantane, and the bottom photo is from a control animal stained for PEG. The graph above shows intensity of staining for PEG in the respective photos.

Conclusion

Addition of an ADM moiety to PEG-HCCs reduces behavioral deficits. This may have occurred because of BBB transport and partial recoupling of endothelial NO synthase, thought to be impaired in hyperglycemic stroke. Further characterization of the range of actions of these materials is underway.

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