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REVIEW PAPER

Oxidative Stress in Alzheimer Disease: Synergy Between the Butterfield and Markesbery Laboratories

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William R. Markesbery, M.D., a 35-year wonderful colleague and deep personal friend of mine, as well as a gifted clinician, neuropathologist, and researcher of Alzheimer disease (AD), died on January 30, 2010. His is a legacy of excellence in all aspects of his life, and a tribute memorial article to his life has been recently published (Butterfield 2010). Bill and I together were among the first to develop the notion that oxidative stress in brain was associated with AD and arguably its earliest form, mild cognitive impairment (MCI), and that oxidative stress may underlie the progression of MCI to AD (Smith et al. 2001; Hensley et al. 1995; Markesbery 1997; Butterfield and Lauderback 2002; Butterfield et al. 2001, 2002, 2003, 2006a, 2007a, b; Keller et al. 2005; Castegna et al. 2002a, b, 2003; Butterfield 2004; Sultana et al. 2006a, b, 2007; Perluigi et al. 2009; Reed et al. 2008).

Oxidative stress is the imbalance between free radical production and free radical scavenging (Butterfield and Stadtman 1997). Butterfield and Markesbery showed that, compared to control, brain in subjects with AD and MCI is under significant oxidative stress, evinced by, among other markers, elevated indices of protein oxidation (protein carbonyls; 3-nitrotyrosine), lipid peroxidation (protein-bound and free 4-hydroxy-2-nonenal {HNE}; F2-isoprostanes (IsoP) and F4-neuroprostanes (NeuroP); other markers), and oxidation of nucleic acids (8-hydroxy-2-deoxyguanosine; 8-hydroxyguanosine, among others) (Smith et al. 2001; Hensley et al. 1995; Markesbery 1997; Butterfield and Lauderback 2002; Butterfield et al. 2001, 2002, 2003, 2006a, b, 2007a, b; Keller et al. 2005; Castegna et al. 2002a, b, 2003; Butterfield 2004; Sultana et al. 2006a, b, 2007; Perluigi et al. 2009; Reed et al. 2008; Markesbery and Lovell 1998; Lovell et al. 2001; Reich et al. 2001; Lauderback et al. 2001; Markesbery and Lovell 2006). Other laboratories also have reported markers of oxidative stress in AD and MCI brain, but because this memorial article deals only with such research conducted in the Butterfield and Markesbery laboratories, no offense is intended for not mentioning others' research in oxidative stress in these disorders.

Earl Stadtman and Rodney Levine developed a means of measuring protein oxidation employing spectroscopic determination of the 2,4-dinitrophenylhydrozone adduct of protein carbonyls (Levine et al. 1990). Bill Markesbery joined Stadtman to use this technique to show that protein oxidation was present in AD brain (Smith et al. 2001), while our laboratory collaborated with the Markesbery laboratory to show that protein oxidation occurred in brain regions that correlated with major markers of histopathology in AD (Hensley et al. 1995), i.e., protein oxidation was found in brain regions rich in amyloid β-peptide (Aβ), but not in Aβ-poor cerebellum. Additional studies of protein oxidation in AD and amnestic MCI have been published from the collaboration between the Butterfield and Markesbery laboratories (Butterfield and Lauderback 2002; Butterfield et al. 2001, 2002, 2003, 2004, 2006a, b, 2007a, b; Keller et al. 2005; Castegna et al. 2002a, b, 2003; Sultana et al. 2006a, b, 2007; Perluigi et al. 2009; Reed et al. 2008).

Studies from our laboratory conducted with Markesbery and Mark Mattson showed that Aβ itself was associated with protein oxidation in neuronal culture in vitro that could be blocked by the chain-breaking antioxidant, vitamin E, or other antioxidants (Yatin et al. 1999b, 2000; Boyd-Kimball et al. 2004; Mark et al. 1995; Kanski et al.

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2002; Varadarajan et al. 2001; Sultana et al. 2005; Aksenov et al. 2000, 2001), and in vivo oxidative stress associated with Aβ was found, consistent with the notion that this peptide plays critical roles in AD and MCI (Yatin et al. 1999a; Drake et al. 2003; Boyd-Kimball et al. 2005, 2006; Mohmmad-Abdul et al. 2006, 2008; Butterfield et al. 2010).

Using redox proteomics, the Butterfield laboratory in collaboration with the Markesbery laboratory identified more than 40 oxidatively modified brain proteins in AD and MCI brain, each of which was consistent with the biochemistry, pathology, and/or clinical presentation of these disorders (Butterfield et al. 2006a; Castegna et al. 2002a, b, 2003; Butterfield et al. 2003, 2004; Sultana et al. 2006a, b, 2007; Perluigi et al. 2009; Reed et al. 2008).

Markesbery showed elevated free levels of the lipid peroxidation products, HNE, acrolein, IsoP, and NeuroP in AD (Markesbery and Lovell 1998; Lovell et al. 2001; Reich et al. 2001), while our laboratory showed elevated levels of protein-bound HNE in AD and MCI (Perluigi et al. 2009; Reed et al. 2008; Lauderback et al. 2001; Butterfield et al. 2006b).

This legacy of research synergy between a neurochemist and a neuropathologist is itself a testament to the closeness with which Bill Markesbery and I worked, thought, had funded grants together, etc. However, there was much more to this relationship than just professional accomplishment; there also was a deep friendship. It is to this friendship that the remainder of this article is dedicated.

I could wax long about each of Bill's many and profound professional accomplishments, such as his more than 400 scientific papers published, his distinguished record of NIH grant funding, his insights into the cause and treatment of Alzheimer disease, including his strong record of studies related to oxidative stress in AD brain noted above, and his recognition by the AD research community as being named the 23rd most influential and cited AD researcher in the world, as well as his being honored with the Zavan Katchaturian Award from the National Alzheimer's Association last year.

However, I would like to write briefly about my friend and colleague on a more personal level. As mentioned previously, Bill and I published many papers together, and we had NIH grants together for many years. We were close, good friends, and I worried about him after he became ill.

Even though many of us agonized over his health for some time, he surprised us. He was engaged in his life's work, and in spite of illness, he set the pace for the rest of us. I have been fortunate to be associated in my professional life with many hard-working persons, including many reading this article. But Bill Markesbery went beyond hard working. Yes, he was innovative; yes, he was a prolific researcher, but what I keep coming back to, as I miss and remember him, is that he was extraordinarily kind. I became a better human being when I was around him. I listened better because he listened intently to me. I spoke more thoughtfully because he spoke thoughtfully to me. I am sure that I am not the only person who had this experience in Bill's company.

We all try to bring the dimensions of efficiency, achievement, and important results to our work; Bill did all that but under the umbrella of kindness and humility and an unusual sense of responsibility that went well beyond anything that benefitted him personally and professionally. He brought the dimensions of ethics and thoughtfulness to matters of process and to the treatment of patients and families with Alzheimer disease. Bill wore his "beeper," knowing that at a moment's notice he could be called day or night to the University of Kentucky (UK) for an autopsy on a subject with AD or for diagnosis of a glioma for a patient on the operating table. In all cases, he performed this duty with a profound knowledge that his skills would help others. I would characterize his manner and legacy as "a life of professional excellence coupled with kindness and humility." Would that we all could be so remembered.

Bill welcomed me to UK in 1975, just 2 weeks after I arrived from Duke University. He said he knew of my graduate and postdoctoral research there and asked me to come and meet him. We became instant and life-long friends. Bill provided counsel, mentoring, resources, and just plain Kentucky good advice to help me along my professional career. There is no way to repay someone directly for their extraordinarily kind approach, so the only thing I could do was try to be like Bill in helping my own students and postdocs, as well as junior colleagues to succeed just as Bill had helped me. I always will be grateful to Bill Markesbery for his deep personal friendship.

And it is in the role of friend that I would ask you, the reader, to please indulge me for a much different and lighter remembrance: As many of you know, in addition to being an internationally recognized researcher in the AD field, Bill was a sports fanatic. He loved his UK Wildcats, and like most Kentuckians he lived and agonized by UK wins and losses in basketball and other sports. In fact, he was a walk-on for UK legendary basketball coach, Adolph Rupp. I know, for sure, from our many long conversations that the focal points of his life were his wife, Barbara, his children Susanne, Kendall, and Allison, and his wonderful grandchildren.

We talked about and planned many things scientific, but also talked about our families, politics, and of course UK sports. Sometimes in the midst of great seriousness, people need time to be a bit goofy, and Bill did this as well. On one occasion I vividly remember his words to me: "Allan: your research in Alzheimer disease is outstanding and

brings great visibility to the University of Kentucky and hope to patients and families with this terrible disease." I think to myself: "Wow, this is so great to be hearing these words from such a distinguished person in this field of research." Then Bill, with a serious look on his face, but a twinkle in his eye said, "There is, however, one problem with you: namely, you went to Duke, and every time UK and Duke play basketball against each other, I start to wonder, where are your loyalties?" We would always have a good chuckle together when he would bring up this topic.

This story assumes you know that UK and Duke are fierce rivals in basketball, but, it turns out, also rivals in neuroscience research. Bill's vision and legacy in conceiving and fostering the Sanders-Brown Center on Aging and the Alzheimer Disease Clinical Center will enable UK to remain a top-ranked institution for Alzheimer disease research.

In the days and developments that surely lie ahead, building on Bill's profound legacy of excellence in the field of Alzheimer disease research, yes, Bill will be remembered for his extraordinary accomplishments, but I, and many more of us, also will remember him for his vision, his kindness and his generosity.

I am so much a better person for having known and worked with Bill Markesbery.

I do and will miss my dear friend and colleague terribly.

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