

OXIDATIVE PROCESSES IN THE BRAIN AND NON-NEURONAL TISSUES AS BIOMARKERS OF ALZHEIMER'S DISEASE

Gary E. Gibson and Hsueh-Meei Huang

Weill Medical College of Cornell University at Burke Medical Research Institute, 785 Mamaroneck Avenue, White Plains, NY 10605

TABLE OF CONTENTS

1. Abstract
2. Introduction
 - 2.1. Abnormalities in metabolism and oxidative stress as early indicators of AD
 - 2.2. The use of peripheral tissues in the study of AD
 - 2.3 Comparison of tissues to test for non-neuronal changes in AD
3. Measures in cerebrospinal fluid (CSF) as markers of AD-related changes in metabolism and oxidative stress
4. AD-related changes in plasma/serum as markers of altered metabolism and increased oxidative stress
5. Measurements in urine as evidence of AD-related oxidative stress
6. Abnormalities in metabolism and oxidative stress in platelets from AD patients
7. Red blood cells and AD-related changes in metabolism/oxidative stress
8. Results with cybrids reveal a relation of oxidative stress/metabolism to AD
9. Lymphocytes reflect abnormalities in oxidative stress/metabolism in AD
10. Changes in lymphoblasts suggest oxidative abnormalities accompany AD
11. Changes in fibroblasts suggest that oxidative stress and abnormal metabolism accompanies AD
12. Use of peripheral tissues to study mechanisms and potential therapies
13. Perspective
14. Acknowledgements
15. References

1. ABSTRACT

Diminished metabolism and excessive oxidative stress occur in the brains of patients with Alzheimer's Disease (AD). These abnormalities in oxidative processes occur in the brain in early stages of AD, which suggests that the deficits are not just secondary to the neuro-degeneration. Alterations in oxidative processes also occur in early stages of AD in non-neuronal tissues including fluids (e.g., cerebrospinal fluid, plasma and urine), cell like particles (e.g., red blood cells and platelets) and cells (e.g., lymphocytes). AD-related abnormalities also persist in cultured cells such as fibroblasts, which indicates that the AD-related changes are not secondary to pathology, and reflect inherent properties of AD cells. These measures of abnormalities in oxidative processes in peripheral cells from AD patients have the potential to be useful as diagnostic markers, as indicators of the progression of the disease, as a tool to develop therapeutic approaches and as monitors of therapeutic efficacy. The peripheral cells are also useful for discovering mechanisms that underlie the multiple changes in cell signaling pathways that accom-

pany AD. Several experimental approaches suggest that oxidative stress is a convergence factor that leads to many other AD-related changes. This review focuses on the considerable recent progress in the quest for markers of metabolism/oxidative stress in peripheral tissues from AD patients, and on experiments to test their pathophysiological importance.

2. INTRODUCTION

2.1. Abnormalities in metabolism and oxidative stress as early indicators of AD

Oxidative stress and abnormalities in glucose metabolism likely have a major role in the pathogenesis of Alzheimer's disease (AD). Studies in brain provide overwhelming evidence that glucose metabolism by the brains of AD patients is diminished, and that the brains of AD patients are undergoing excessive oxidative stress. These changes have been reviewed extensively (1-6). Whether these abnormalities cause, or result from, the

Table 1. Peripheral tissues that have been used to examine the role of abnormalities in metabolism and oxidative stress in AD

Fluids	
	• CSF • Serum • Plasma • Urine
“Cell like” components	
	• Platelets • Red blood cells
Cybrids	
Cells	
	• Lymphocytes • Transformed lymphocytes (i.e., lymphoblasts) • Fibroblasts

pathology is not practical to decipher in human brain because of the difficulty of evaluating temporal changes of critical variables in living patients. Although temporal changes in metabolism in human brain can be accurately assessed, sequential evaluations of brain pathology or oxidative stress is not possible. Nevertheless, recent results provide strong evidence that altered metabolism and oxidative stress precede the clinical symptoms and the development of pathology.

Recent studies demonstrate that deficits in glucose metabolism and abnormalities in oxidative stress occur in early stages of AD. Glucose utilization, as determined by 2-¹⁸F)fluoro-2-deoxy-d-glucose/positron-emission tomography, was diminished in 25% of a healthy, elderly population. In this population, reductions in glucose utilization in the entorhinal cortex predict subsequent involvement of the hippocampus and neocortex, and accurately predict the conversion from normal cognition to “mildly cognitively impaired”. Among those subjects who decline, apolipoprotein E4 carriers, a known risk factor for non-genetic forms of AD, show marked longitudinal reductions in temporal neocortex. Thus, a decline in glucose metabolism in normal elderly predicts future reductions in cognition and brain metabolism (7). A second study examined the relation of oxidative stress to the pathology of AD in transgenic mice. These experiments utilized transgenic mice, because making temporal measures of pathology and oxidative stress in humans is not feasible. The results demonstrate that measures of oxidative stress in the periphery precede the formation of plaques in the brain, a hallmark of AD. Isoprostanes, a common measure of oxidative stress, increase in the urine and plasma before plaques occur in the brains of these transgenic mice. This provides strong evidence that oxidative stress is an early event in AD, and that changes occur in the peripheral tissues as well as in the brain. Peripheral measures, unlike those in brain can be monitored to determine the extent of the disease and perhaps to monitor therapeutic efficacy (8).

2.2. The use of peripheral tissues in the study of AD

Considerable evidence suggests that AD may not simply be a brain disease, but that changes occur in

peripheral tissues as well. The presence of replicable, AD-specific changes in non-CNS tissues would be important for understanding the mechanism(s) leading to the disease and for the development of new therapeutic approaches. The changes in peripheral tissues that mimic those in the CNS would suggest that the alterations in brain are not secondary to neurodegeneration, but reflect inherent abnormalities in the cells that may lead to the neurodegeneration. The changes in peripheral tissues could be used to diagnose the disease, to determine the precise nature of the oxidative damage (i.e., the nature of the radicals), to study the origin of these species, and to develop therapeutic approaches. The development of efficient methods for examining a large number of genes has created another potentially valuable use of peripheral cells in AD research. Since these cell populations are homogeneous compared to brain, it is possible to screen them for unknown gene defects that are altered (or induced) by AD (9). Although several measures of oxidative stress and metabolism have been reported in the periphery, none has proven to be diagnostic, and few have been replicated in multiple laboratories. This review updates our previous overview on changes in oxidative processes and metabolism in non-neuronal tissues of AD patients (10). In general, the state of the field at the time of the previous review (10) is presented, and new findings that were reported in 2001 are reviewed in detail.

2.3. Comparison of tissues to test for non-neuronal changes in AD

A variety of tissues have been used to test for AD-related changes in metabolism and oxidative stress (Table 1). Cerebrospinal fluid (CSF) bathes the brain, so it likely reflects the changes in the brain better than other tissues. CSF samples also reflect changes in the spinal cord. However, the movement of CSF from brain to site of sampling requires considerable time, and samples of CSF are relatively difficult to obtain. On the other hand, plasma/serum, urine, platelets and red blood cells are readily accessible. However, the changes in these tissues also reflect the patients’ diet and/or drugs, and cannot be used to study cell properties. Abnormalities can reflect either acute (e.g., the presence of drugs) or chronic changes (e.g., changes in membranes due to diet) in oxidation. Platelets can either be studied directly or by transferring mitochondrial DNA from the platelets of the living patients into neuroblastoma cells to create cybrids. The disadvantage of cybrids is that the cells have a different nuclear genetic background than the patients, and the phenotypes return to normal across time. Lymphocytes have the advantage that they are cells, so that cellular signaling properties and the interaction of the patients’ genome with mitochondria and oxidative stress can be assessed. However, lymphocytes reflect the patients’ diets and drugs, because they are generally used immediately after isolation from the patient. Lymphoblasts have the advantage that the effects of the patients’ drugs and diets have little influence because the cells are maintained in culture. However, lymphoblasts are transformed cells, which have many abnormal cellular properties. Fibroblasts are non-transformed cells with the advantages of being maintained

Table 2. Important properties that may compromise the use or interpretation of peripheral tissues in the study of abnormal metabolism or oxidative stress

-
- Accessibility
 - Presence of the patients' mitochondria
 - Presence of patients' nuclear genome
 - Non-transformed cells
 - Minimization of drug or diet effects
 - Ability (and possibility) to reproduce measures between laboratories
-

in culture. No marker of AD-related abnormalities in oxidative stress has been identified in all of these tissues. The qualities that are desirable in tissues that will be used to examine peripheral markers are summarized in Table 2. A marker in the body fluids that is disease specific would obviously be helpful. However, cells (or platelets) that actually contain mitochondria are more helpful for mechanistic studies, and for the development of therapeutic regimens related to changes in oxidative processes.

One difficulty that has slowed the use of peripheral tissues in AD research is the lack of reproducibility between laboratories. Determining the same measure on the same sample assures that a technical difference does not account for different reports on various patient populations. However, repeat measures on some tissues such as CSF from a single patient is difficult because of the sample availability. Cultured cells such as lymphoblasts or fibroblasts are particularly advantageous in this regard because they can be produced in large quantities, stored in liquid nitrogen, and then shipped to laboratories all over the world. This provides a large supply of essentially identical cells that can be studied in multiple laboratories.

3. MEASURES IN CSF AS MARKERS OF AD-RELATED CHANGES IN METABOLISM AND OXIDATIVE STRESS

Measurements in the CSF support the suggestion that the AD brain is undergoing considerable oxidative stress. Previous results on CSF suggest that the levels of antioxidants are lower in CSF of AD subjects, and that proteins, nucleic acids and lipids show evidence of ongoing oxidative stress (10). New results confirm and extend those findings. For example, concentrations of melatonin, a potent antioxidant, decreases in CSF of AD patients (11). Previously reported measures of oxidative stress in CSF of AD patients overlapped with that from control subjects. The ratio of 8-hydroxyguanine (8-OHG) levels in intact DNA to free 8-OHG in the ventricular CSF of patients provides a sensitive measure of DNA oxidation. Recent results suggest a statistically significant 108-fold increase in the ratio of 8-OHG in intact DNA to free 8-OHG in patients with AD. The lowest AD ratio is 3.5 times higher than the highest control ratio and the two populations do not overlap. Thus, the ratio of 8-OHG intact in DNA to free 8-OHG delineates between patients with AD and control subjects, and may be useful as a marker of disease progression or the efficacy of therapeutic antioxidant intervention (12). Not only nucleic acids but also lipids show evidence of oxidative damage. Isoprostanes,

prostaglandin isomers formed by peroxidation of polyunsaturated fatty acids, are elevated in CSF of AD patients (13). The increase in isoprostanes is correlated to the severity of the dementia, to increases in CSF tau and the decrease in CSF amyloid beta peptide1-42 (13). Changes in the level of an early glycation product in CSF were studied with ageing and in late-onset AD. The level of CSF glycation product is 1.7-fold higher in AD patients as compared with non-demented age-matched control group. An increased accumulation of glycation products is found in all major proteins of CSF of AD including albumin, apolipoprotein E and transthyretin. The increased early glycation of CSF proteins in the AD patients may stimulate the formation and the consequent deposition of advanced glycation end products as well as oxidative stress in the brain (14). Together these new results enhance the conclusion that during AD, oxidative stress damages macromolecules in the CSF, and that the ability to remove reactive oxygen species (ROS) (i.e., antioxidant capacity) is diminished.

Activation of microglia and astrocytes can also lead to oxidative stress in brain. To test whether compounds in the CSF from AD patients can activate these cells, CSF from AD patients was added to rat microglia and astrocytes. The concentration of nitric oxide or interleukin-6 (i.e., two measures of activation) is not increased by CSF from AD patients. Whether other proinflammatory cytokines are activated is unknown. These findings suggest that the stimuli for inflammatory activation of glia in brain are quite localized, and are not present in sufficient concentrations in the CSF of affected patients (15).

4. AD-RELATED CHANGES IN PLASMA/SERUM AS MARKERS OF ALTERED METABOLISM AND INCREASED OXIDATIVE STRESS

Plasma provides a readily accessible tissue to monitor the response to oxidative stress. Plasma reflects multiple aspects of all of the organs of the body: nutrition, drugs, stress, time of day that samples are taken, time after eating, etc. This may explain why the results on measures of oxidative stress/metabolism in plasma are highly variable between laboratories. No reports exist in which multiple groups have examined the same samples to determine if apparent discrepancies are technical, or true differences between patient populations. In general, previous studies show that in AD plasma concentrations of antioxidants are diminished while oxidized species increase (10).

Between laboratory discrepancies persist in recently published results. One study compares markers of oxidative stress in carefully characterized control and AD populations. Both groups include normally nourished elderly people living at home, free from disease and not undergoing any treatment that influences oxidative stress markers or antioxidant defense systems. The groups are similar in age, body mass index, dietary record and serum albumin concentration. After adjustment for age, sex and cardiovascular co-morbidity, mean plasma concentrations of alpha-tocopherol and retinol are about 20% lower in AD

subjects than in controls. On the other hand, the mean concentration of free plasma malondialdehyde in this same population is about 40% higher in subjects with AD. In AD patients, free plasma malondialdehyde concentrations correlate inversely with levels of alpha-tocopherol and retinol. The lower plasma concentrations of alpha-tocopherol and retinol in the AD patients suggest that these antioxidant vitamins had been consumed as a result of excessive production of free radicals (16). A second study of a carefully selected population reports no differences in malondialdehyde (17). However, in this population another oxidized species, 4-hydroxynonenal (4-HNE), is about 2.5 times higher in plasma from AD patients compared to controls, and levels of ascorbate are reduced by more than half in the AD group. In this population, the levels of 4-HNE in AD patients are inversely related to ascorbate and to the Folstein Mini-Mental State Examination. The concentration of protein sulphydryls, which are free-radical scavengers, are also directly related to the Mini-Mental State Examination scores. (17). Another study reports that isoprostanes, prostaglandin isomers formed by free radical peroxidation of polyunsaturated fatty acids, are elevated in plasma (13). Thus, recent studies in plasma confirm that the concentrations of antioxidants are lower, and compounds produced by ROS are increased. However, which compounds are found to be altered varies between laboratories.

Plasma measures are more reliable in transgenic mice because animals' diets. Drugs and behavior are well controlled. Plasma levels of isoprostanes (i.e., 8,12-iso-iPF(2alpha)-VI) are elevated as early as 8 months of age in transgenic animal models of plaque formation. In these same mice, amyloid- β peptide deposits do not occur until 12 months of age (13). These studies suggest that oxidative stress in plasma precedes plaque formation in brain in AD patients, and that the changes in plasma isoprostanes could be used as an endpoint to monitor therapeutic efficacy.

5. MEASUREMENTS IN URINE AS EVIDENCE OF AD-RELATED OXIDATIVE STRESS

Oxidized metabolites can be readily measured in urine, and these are elevated in AD patients compared to controls. Increases in the isoprostane iPF(2alpha)-III in the urine of patients with mild to moderate dementia are associated with probable AD compared to non-demented subjects (13,18). 2,3-Dinor thromboxane B(2) (dinor TXB(2)), a urinary metabolite of TXB(2), an indicator of the enzymatic transformation of a product of arachidonic acid, is also elevated in AD patients with dementia and probable AD as compared to the non-demented subjects. Values of iPF(2alpha)-III and dinor TXB obtained for demented and non-demented patients overlap (2). Increases in urine prostanes also precede plaque formation in the brain in transgenic animal models of plaque formation. Levels of urinary 8,12-iso-iPF(2alpha)-VI increase in urine months before plaques are formed in the brain (8). These results suggest that 8,12-iso-iPF2alpha-VI is a useful biomarker of oxidative damage in AD (8,13).

Glucocorticoids can promote cell death. Glucocorticoid production is nearly three times higher in AD

versus healthy elderly control subjects. The elevated concentration is related to an increase in glucocorticoid production, which is an early feature of AD (19).

6. ABNORMALITIES IN METABOLISM AND OXIDATIVE STRESS IN PLATELETS FROM AD PATIENTS

Metabolic changes in platelets are well known, and current studies focus on the role of oxidative stress or altered metabolism on the processing of amyloid precursor protein (APP). Cytochrome c oxidase, but not complex III (ubiquinol:cytochrome c oxidoreductase) or complex II (succinic dehydrogenase), is reduced in platelets from patients with AD (20). Platelets and neurons both contain large quantities of two carboxyl-truncated 120 to 130 and 110 kDa APPs. Platelets from AD patients contain a reduced ratio of these APPs. The accuracy of the ratio to identify AD is high. Decreased values are found throughout the course of AD, and are associated with severity of symptoms. Some results suggest that platelet APP ratio is a potential clinical marker because the ratio patterns become more control like with effective treatments (21,22). The release of secreted beta-amyloid precursor protein (AbetaPPs) in response to thrombin stimulation in platelets is sensitive to oxidative stress and/or altered metabolism. Thrombin produces a concentration-dependent release of AbetaPPs with a concomitant reduction in the AbetaPP remaining in the platelet lysates. The response to thrombin is not affected by pretreatment with hydrogen peroxide. In contrast, pretreatment with azide, which blocks cytochrome oxidase, or 4-HNE reduces the responses of Abeta PP to thrombin (23). Thus, platelets can be used to test the interaction of oxidative processes with amyloid processing.

Calcium homeostasis is altered in platelets from AD patients, has been implicated in the neuropathogenesis of AD, and is sensitive to oxidative stress and altered metabolism. Whether changes in cytosolic calcium level ($[Ca^{2+}]_i$) are the result or the cause of pathogenic effects is not clear. The basal values of $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+} are significantly lower in platelets of patients with early stages of AD than controls. The addition of calcium to the incubation medium markedly increases $[Ca^{2+}]_i$ in platelets of AD patients compared to controls. The results suggest that the disturbed calcium homeostasis in AD is an "early defect." (24). Calcium responses are sensitive to metabolic inhibitors such as azide, which impairs the respiratory chain, and increases ROS production. Azide reduces the Ca^{2+} response to serotonin without a corresponding reduction in the responses to thrombin or beta-amyloid(25-35). In contrast, the effect of azide upon the response to serotonin is not blocked by glutathione or dithiothreitol. The Ca^{2+} response to thrombin is greatly reduced by azide, but not by 4-HNE (25).

7. RED BLOOD CELLS AND AD-RELATED CHANGES IN METABOLISM/OXIDATIVE STRESS

Reports on changes in oxidants/metabolism in red blood cells are variable. They demonstrate that changes in oxidative processes can be measured in red blood cells, but

which variable changes in which direction varies between laboratories and/or patient populations, and none of the variables are diagnostic for AD. For example, one study suggests that erythrocyte levels of antioxidant vitamins (alpha-tocopherol, retinol) and erythrocyte enzymatic activities of glutathione peroxidase and copper-zinc superoxide dismutase are similar in the AD and control groups (16). On the other hand, a second report suggests that superoxide dismutase activities are higher in AD patients than controls, while catalase, glutathione and thiobarbituric acid reactive substances are similar in the two groups (26).

8. RESULTS WITH CYBRIDS REVEAL A RELATION OF OXIDATIVE STRESS/METABOLISM TO AD

Transferring mitochondria from platelets obtained from disease and control donors into mitochondrial DNA-depleted recipient neuron-based cells (rho 0 cells) creates cytoplasmic hybrid (cybrid) cells where the mitochondrial DNA (mtDNA) from the donor is expressed in the nuclear and cellular background of the host rho 0 cell. Any differences reflect the mitochondrial DNA of platelets from the patients. Previous reports indicate that cybrids from AD patients have reduced activity of complex IV, as well as altered homeostasis of calcium and ROS. Recent studies indicate that AD cybrids are more sensitive to apoptotic oxidative stress (H_2O_2) and have dysfunctional nerve growth factor metabolism (27). Furthermore, the average velocity of mitochondrial movement is reduced about 25% in AD cybrids. The AD cybrids also have fewer and more elongated mitochondria than controls (28).

9. LYMPHOCYTES REFLECT ABNORMALITIES IN OXIDATIVE STRESS/METABOLISM IN AD

Previous studies of lymphocytes demonstrate that elevated oxidative stress occurs in cells from patients with AD, and that AD cells are more sensitive to irradiation-induced damage. Recent studies focus on the functional implication of those changes. Apoptotic cell death is often considered to be a likely mechanism of cell death in AD. The vulnerability of lymphocytes from AD patients to undergo apoptosis is increased compared to non-demented elderly controls and relative to patients with subcortical vascular encephalopathy as another, but demented, control group. Quiescent 'native' and 'activated' lymphocytes from AD patients, which were predisposed to commit apoptotic cell death by priming the cells with interleukin-2, accumulate apoptosing cells to a higher extent in spontaneous and in oxidative stress-induced *in vitro* apoptosis. The comparable findings of a higher extent of apoptotic features in neurons and in peripheral blood cells of AD patients suggest a rather general modulation of apoptotic mechanisms by the disease (29). Lymphocytes from PS1 mutant transgenic mice show a similar hypersensitivity to cell death as do peripheral cells from AD patients and several cell culture systems expressing PS1 mutations. The enhancement of cell death by PS-1 mutation is associated with increased production of ROS and altered calcium regulation, but not with changes in mitochondrial cytochrome c (30). Thus, several lines of evidence suggest

that lymphocytes from AD patients have abnormalities in their ability to handle oxidative stress and this promotes apoptosis.

10. CHANGES IN LYMPHOBLASTS THAT SUGGEST OXIDATIVE ABNORMALITIES ACCOMPANY AD

Studies of lymphoblasts, immortalized lymphocytes, support the suggestion that an altered ability to handle oxidative stress is an inherent property of cells from patients with AD. The use of these cultured cells has the advantage that any effect is not secondary to the patients' diet or drugs. Previous studies with lymphoblasts found AD-related differences in glutathione metabolism, and a diminished ability to repair DNA, but none of the differences were diagnostic for AD. The development of efficient methods for examining a large number of genes has created another valuable use of peripheral cells in AD research. Since these cells are homogeneous compared to brain, lymphoblasts can be used to screen for unknown gene defects that alter (or induce) AD (9). Marked depletion of mRNA of one member of the apoE/low density lipoprotein receptor family (LR11, SorLA) was discovered in AD lymphocytes. Subsequent testing in brain homogenates confirmed that the mRNA is also severely depleted in brain. The large numbers of genes involved in oxidative metabolism and the cells ability to handle oxidative stress have not been studied by this powerful approach.

11. CHANGES IN FIBROBLASTS SUGGEST THAT OXIDATIVE STRESS AND ABNORMAL METABOLISM ACCOMPANIES AD

Fibroblasts provide several advantages for testing whether cells from AD patients have an altered ability to handle oxidative stress. They have same genetic background as the patient. They have normal levels of any mutant protein, and the expression of the mutant protein is under normal cellular controls. This is important because the genetic background of cells and level of the protein alter the effects of AD-producing mutations. Fibroblasts have not been transformed, which can alter cellular signaling. Fibroblasts are maintained in culture so that any contributions from patient's blood, nutrition, drugs, stress or products of neurodegeneration are diluted millions of times. Any finding can be tested by multiple laboratories using the same cells, since large quantities of these cells can be frozen and shipped all over the world (31-34). These cells can be used for studies on mechanism. Thus, the use of fibroblasts appears to be a particularly valuable approach for testing whether oxidative stress is secondary to neurodegeneration.

A comparison of the ability of control and AD fibroblasts to respond to oxidative stress provides a test of whether an altered ability to handle oxidative stress is an inherent property of AD cells. Basal (-10%) and H_2O_2 (-16%) stimulated levels of ROS are only slightly lower in cells from AD subjects than controls. However, treatments with antioxidants reveal clear differences. Pretreatment with DMSO, a scavenger of hydroxyl radicals, reduces

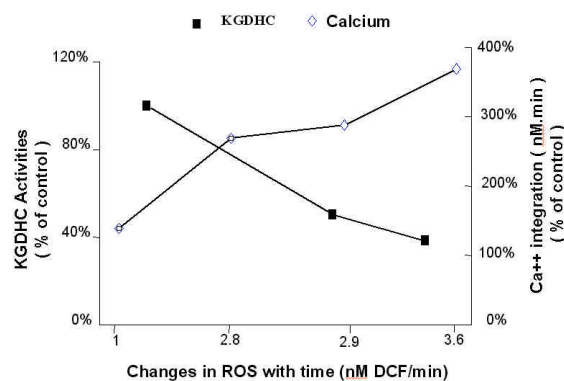


Figure 1. H₂O₂-induced ROS diminishes KGDHC and exaggerates bombesin-releasable calcium stores. Fibroblasts were treated with various concentrations of H₂O₂. The increase in ROS was monitored with cH₂DCF. Internal calcium stores were determined by adding low concentrations of bombesin. KGDHC was determined in cell lysates after treatment with H₂O₂ (35).

basal and H₂O₂-induced ROS levels significantly more in cells from controls (-22%, -22%) than in those from AD subjects (-4%, +14%). On the other hand, pretreatment with Trolox diminishes H₂O₂-induced ROS significantly more in cells from AD (-60%) than control (-39%) subjects. Thus, cells from AD patients have greater Trolox-sensitive ROS and less DMSO-sensitive ROS than controls. The results demonstrate that fibroblasts from AD patients respond to stress differently than controls, and appear useful for determining the mechanism underlying the altered redox metabolism (35).

Fibroblasts from AD patients are more sensitive than controls to ROS, and ongoing studies are beginning to define which reactive species may be involved. Fibroblasts from familial AD patients are more sensitive than control fibroblasts to oxygen metabolites generated by the enzymatic oxidation of acetaldehyde (36). Iron increases calcium uptake more in cells from AD patients than in cells from controls. Pretreatment with U-74500A, or desferoxamine, prior to exposure to iron, is able to completely protect control mitochondria, but only partially protect AD mitochondria (37). The levels of free radical scavenging enzymes do not mediate these differences. Fibroblast SOD- and SOD mRNA levels are significantly higher in AD patients over 65 years of age, while they are lower in patients under 65 years (38).

Measures of cell survival and DNA repair in response to a variety of treatments that generate oxidative stress also show that fibroblasts from AD patients respond differently to stress than controls. AD cells, unlike normal cells, are slow, or fail, to repair DNA damage from methylmethane sulfonate (39), N-methyl-N'-nitro-N-nitrosoguanidine (40) or varying concentrations of alkylating agents (41). Fibroblasts from AD patients have abnormal DNA repair mechanism for damage due to irradiation, beta cytosine arabinoside or caffeine (42). Taken together the results suggest that AD cells have an altered ability to repair DNA following oxidative insults. Trippi et al. (43)

evaluated spontaneous and chemically-induced cytogenetic alterations by means of the micronucleus test in skin fibroblasts of patients with non-genetic or familial AD. The spontaneous micronucleus frequencies of sporadic and familial AD patients are nearly three times higher than those of the control groups. On the other hand, chemical-induced increases in the spontaneous micronucleus frequency of somatic cells were less in all AD patients compared with the control group (43).

Fibroblasts from AD patients are also more sensitive to inhibition of metabolism. Although aglycemia reduces sAPP release in the medium of both AD and control fibroblasts to a similar extent, treatment with azide under glucose deprivation inhibits sAPP secretion from AD fibroblasts, but does not affect sAPP secretion from control fibroblasts (44).

12. USE OF PERIPHERAL TISSUES TO STUDY MECHANISMS AND POTENTIAL THERAPIES

Another related use of peripheral cells is to use them to discover the mechanisms underlying AD-related abnormalities that are present in the cells. The advantage of using peripheral cells to examine mechanisms is that the studies can be done in the genetic background of the patient rather than in a cell that has a totally different genetic background (e.g., cells transfected with AD-causing mutations). The genetic background does modify the response of cells that over express the mutant protein. Fibroblasts from patients with genetic and non-genetic forms of AD show many abnormalities. These changes have been reviewed previously (31,32,33). The increased bombesin-releasable calcium stores (BRCS), diminished activities of the mitochondrial α -ketoglutarate dehydrogenase complex (KGDHC), and an abnormal response to oxidative stress are characteristic of AD cells. The link between genetic mutations (and the unknown primary event in non-genetic forms) and these other cellular abnormalities is unknown. To determine whether oxidative stress could be a convergence point that produces the other AD-related changes, experiments tested in fibroblasts the effects of H₂O₂, in the presence or absence of select antioxidants, on BRCS and KGDHC. H₂O₂ concentrations that elevate carboxy-dichlorofluorescein (cH₂DCF) detectable ROS increase BRCS and decrease KGDHC activity (35). These changes are in the same direction as those in fibroblasts from AD patients (Figure 1). In addition, recent studies indicate that exaggeration of this same calcium pool leads to over activation of ERK1/2 in AD fibroblasts (45).

Correction of these abnormalities by a compound(s) would suggest the compound(s) could be effective therapeutic agents. Acute treatments of fibroblasts with the antioxidants Trolox, or DMSO decrease cH₂DCF-detectable ROS by about 90%, but exaggerate the H₂O₂-induced increases in BRCS by about four-fold, and do not alter the reduction in KGDHC. Chronic pretreatments with Trolox more than double the BRCS, triple KGDHC activities, and reduce the effects of H₂O₂. Thus, Trolox improves one AD-like effect induced by oxidative stress, but exaggerates one of the other effects. Pretreatment with DMSO or N-

acetylcysteine diminishes the BRCS and either has no effect, or exaggerates the H₂O₂-induced changes in these variables. The results demonstrate that BRCS and KGDHC are more sensitive to H₂O₂-derived species than cH₂DCF, and that oxidized derivatives of the antioxidants exaggerate the actions of H₂O₂. The findings support the hypothesis that select abnormalities in oxidative processes are a critical part of a cascade that leads to the cellular abnormalities in cells from AD patients. They also indicate that common antioxidants cannot correct AD related abnormalities in these signals.

Changes in oxidative stress can also be convincingly related to formation of plaques and tangles. Changes in oxidation lead to tau hyperphosphorylation (46), dimerization, and polymerization into filaments (47), which are associated with tangle formation. Changes in oxidation also promote production of amyloid beta-peptide, which promotes plaque formation (48,49). Thus, considerable data are consistent with the idea that the ability to handle oxidative stress is the underlying cause of other abnormalities in AD (i.e., a convergence factor). Thus, appropriate measures of abnormalities in oxidative processes may be good diagnostic markers.

13. PERSPECTIVE

Overwhelming evidence indicates that brains of AD patients have reduced metabolism and are experiencing exaggerated oxidative stress. Measurements in a variety of non-brain tissues verify that AD patients are undergoing oxidative stress and diminished metabolism. Several reports suggest that these peripheral markers may monitor the severity of the disease, and can be used as endpoints to monitor therapeutic efficacy. Experiments on patients' cells that are maintained in culture provide a powerful tool to understand the cause and effect of alterations in oxidative stress/metabolism. The results from studies of brain and non-brain tissues strongly suggest that abnormalities in metabolism and the ability to handle oxidative stress are inherent properties of cells from patients with AD and not just secondary to neurodegeneration. Experiments in non-CNS cells suggest that specific forms of oxidative stress may cause the AD-related alterations in signal transduction and metabolism, and that specific antioxidants, or antioxidant enzymes, may ameliorate the abnormalities. Since the cells have the patient's genetic background these cells may be useful to develop patient specific therapeutic approaches.

14. ACKNOWLEDGEMENTS

Supported by National Institutes on Aging grants AG11921 and AG14930.

15. REFERENCES

1. Blass JP & GE Gibson: Cerebrometabolic aspects of delirium in relationship to dementia. *Dement Geriatr Cogn Disord* 10, 335-8(1999)
2. Gibson GE, JP Blass, HM Huang, & GB Freeman: The cellular basis of delirium and its relevance to age-related

- disorders including Alzheimer's disease. *Int Psychogeriatr* 3, 373-95 (1991)
3. Aliev G, MA Smith, D Seyidov, ML Neal, BT Lamb, A Nunomura, EK Gasimov, HV Vinters, G Perry, JC LaManna & RP Friedland: The role of oxidative stress in the pathophysiology of cerebrovascular lesions in Alzheimer's disease. *Brain Pathol* 12, 21-35 (2002)
4. Markesbery WR & JM Carney: Oxidative alterations in Alzheimer's disease. *Brain Pathol*, 9, 133-46 (1999)
5. Smith MA & G Perry: What are the facts and artifacts of the pathogenesis and etiology of Alzheimer disease. *J Chem Neuroanat*, 16, 35-41(1998)
6. Smith MA, CA Rottkamp, A Nunomura, AK Raina & G Perry: Oxidative stress in Alzheimer's disease: *Biochim Biophys Acta*, 1502, 139-44 (2000)
7. de Leon MJ, A Convit, OT Wolf, CY Tarshish, S DeSanti, H Rusinek, W Tsui, E Kandil, AJ Scherer, A Roche, A Imossi, E Thorn, M Bobinski, C Caraos, P Lesbre, D Schlyer, J Poirier, B Reisberg, & J Fowler: Prediction of cognitive decline in normal elderly subjects with 2-fluoro-2-deoxy-D-glucose/positron-emission tomography (FDG/PET). *Proc Natl Acad Sci USA* 98, 10966-71 (2001)
8. Pratico D, K Uryu, S Leight, JQ Trojanowski, & VM Lee: Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. *J Neurosci* 21, 4183-7 (2001)
9. Scherzer CR, JJ Lah, M Gearing, CJ Heilman, G Fang, BH Wainer & AI Levey: Genomic screening in Alzheimer's disease: novel apoe receptro Lr11 is down regulated. *Soc Neurosci Abstr* 27, 752.17 (2001)
10. Gibson GE & H Zhang: Abnormalities in oxidative processes in non-neuronal tissues from patients with Alzheimer's disease. *J Alz Dis* 3, 329-38 (2001)
11. Maurizi CP: Alzheimer's disease: roles for mitochondrial damage, the hydroxyl radical, and cerebrospinal fluid deficiency of melatonin. *Med Hypotheses* 57, 156-60 (2001)
12. Lovell MA & WR Markesbery: Ratio of 8-hydroxyguanine in intact DNA to free 8-hydroxyguanine is increased in Alzheimer disease ventricular cerebrospinal fluid. *Arch Neurol* 58, 392-6 (2001)
13. Pratico D, CM Clark, VM Lee, JQ Trojanowski, J Rokach & GA FitzGerald: Increased 8,12-iso-iPF₂α-VI in Alzheimer's disease: correlation of a noninvasive index of lipid peroxidation with disease severity. *Ann Neuro*. 48, 809-12 (2000)
14. Wilms H, P Rosenstiel, J Sievers, G Deuschl, & R Lucius: Cerebrospinal fluid from patients with neurodegenerative and neuroninflammatory diseases: no evidence for rat glial activation in vitro. *Neurosci Lett* 314, 107-10 (2001)
15. Shuvaev VV, I Laffont, JM Serot, J Fujii, N Taniguchi & G Siest: Increased protein glycation in cerebrospinal fluid of Alzheimer's disease. *Neurobiol Aging* 22, 397-402 (2001)
16. Bourdel-Marchasson I, MC Delmas-Beauvieux, E Peuchant, S Richard-Harston, A Decamps, B Reignier, JP Emeriau & M Rainfray: Antioxidant defences and oxidative stress markers in erythrocytes and plasma from normally nourished elderly Alzheimer patients. *Age Ageing* 30, 235-41 (2001)

17. McGrath LT, BM McGleenon, S Brennan, D McColl, S McLroy, & AP Passmore: Increased oxidative stress in Alzheimer's disease as assessed with 4-hydroxynonenal but not malondialdehyde. *Quart J Med* 94, 485-90 (2001)
18. Tuppo EE, LJ Forman, BW Spur, RE Chan-Ting, A Chopra, & TA Cavalieri: Sign of lipid peroxidation as measured in the urine of patients with probable Alzheimer's disease. *Brain Res Bull* 54, 565-8 (2001)
19. Rasmuson SR Andrew, B Nasman, JR Seckl, BR Walker & T Olsson: Increased glucocorticoid production and altered cortisol metabolism in women with mild to moderate Alzheimer's disease. *Biol Psychiatry* 49, 547-52 (2001)
20. Parker WD Jr, N Mahr, CM Filley, JK Parks, D Hughes, DA Young & CM Cullum: Reduced platelet cytochrome c oxidase activity in Alzheimer's disease. *Neurology* 44, 1086-90 (1994)
21. Baskin F, RN Rosenberg, L Iyer, L Hynan, & CM Cullum: Platelet APP isoform ratios correlate with declining cognition in AD. *Neurology* 54, 1907-9 (2000)
22. Padovani A, B Borroni, F Colciaghi, L Pastorino, S Archetti, E Cottini, L Caimi, F Cattabeni, & M Di Luca: Platelet amyloid precursor protein forms in AD: a peripheral diagnostic tool and a pharmacological target. *Mech Ageing Dev* 122, 1997-2004 (2001)
23. Hedin HL, L Nilsson & CJ Fowler: Effects of staurosporine, U-73122, wortmannin, 4-hydroxynonenal and sodium azide upon the release of secreted beta-amyloid precursor protein from human platelets in response to thrombin stimulation *Mol Cell Biochem* 219, 145-52 (2001)
24. Ripovi D, V Platilova, A Strunecka, R Jirak & C Hoschl: Cytosolic calcium alterations in platelets of patients with early stages of Alzheimer's disease. *Neurobiol Aging* 21, 729-34 (2000)
25. Hedin HL, S Eriksson & CJ Fowler: Human platelet calcium mobilisation in response to beta-amyloid (25- 35): buffer dependency and unchanged response in Alzheimer's disease. *Neurochem Int* 38, 145-51 (2001)
26. Serra JA, RO Dominguez, ES de Lustig, EM Guareschi, AL Famulari, EL Bartolome & ER Marschoff: Parkinson's disease is associated with oxidative stress: comparison of peripheral antioxidant profiles in living Parkinson's, Alzheimer's and vascular dementia patients. *J Neural Transm* 108, 1135-48 (2001)
27. Lin W, T Dean-McKinney, PS Neff & JB Tuttle: Alzheimer's disease cybrid cells are sensitive to ROS and have dysfunctional nerve growth factor metabolism. *Soc Neurosci Abstr* 27, 651.8 (2001)
28. Borland MK, JA Almeida & PA Trimmer: Reduced movement of mitochondria in Alzheimer's disease: a study using cybrid cell lines. *Soc Neurosci. Abstr* 27, 651.2 (2001)
29. Eckert A, M Oster, R Zerfass, M Hennerici & WE Muller: Elevated levels of fragmented DNA nucleosomes in native and activated lymphocytes indicate an enhanced sensitivity to apoptosis in sporadic Alzheimer's disease. Specific differences to vascular dementia. *Dement Geriatr Cogn Disord* 12, 98-105 (2001)
30. Eckert A, K Schindowski, S Leutner, C Luckhaus, N Touchet, C Czech & W E Muller: Alzheimer's disease-like alterations in peripheral cells from presenilin-1 transgenic mice. *Neurobiol Dis* 8, 331-42 (2001)
31. Etcheberrigaray E, GE Gibson, & DL Alkon: Molecular mechanisms of memory and the pathophysiology of Alzheimer's disease. *Ann N Y Acad Sci* 747, 245-55 (1994)
32. Gasparini L, M Racchi, G Binetti, M Trabucchi, SB Solerte, D Alkon, R Etcheberrigaray, GE Gibson, J P Blass, R Paoletti & S Govoni: Peripheral markers in testing pathophysiological hypotheses and diagnosing Alzheimer's disease. *FASEB J* 12, 17-34 (1998)
33. Huang HM, R Martins, S Gandy, R Etcheberrigaray, E Ito, DL Alkon, JP Blass, & GE Gibson, Use of cultured fibroblasts in elucidating the pathophysiology and diagnosis of Alzheimer's disease. *Ann N Y Acad Sci* 747, 225-44 (1994)
34. Pascale A & R Etcheberrigaray: Calcium alterations in Alzheimer's disease: pathophysiology, models and therapeutic opportunities. *Pharmacol Res* 39, 81-8 (1999)
35. Gibson GE, H Zhang, KFR Sheu & LC Park: Differential alterations in antioxidant capacity in cells from Alzheimer patients. *Biochim Biophys Acta* 1502, 319-29 (2000)
36. Tesco G, S Latorraca, P Piersanti, S Piacentini, L Amaducci & S Sorbi. Alzheimer skin fibroblasts show increased susceptibility to free radicals. *Mech Ageing Dev* 66, 117-20 (1992)
37. Richardson JS: Free radicals in the genesis of Alzheimer's disease. *Ann N Y Acad Sci* 695, 73-6 (1993)
38. Urakami K, K Sato, A Okada, T Mura, T Shimomura, T Takenaka, Y Wakutani, T Oshima, Y Adachi, K Takahashi: Cu, Zn superoxide dismutase in patients with dementia of the Alzheimer type. *Acta Neurol Scand* 91, 165-8 (1995)
39. Robison SH, JS Munzer, R Tandan & WG Bradley: Alzheimer's disease cells exhibit defective repair of alkylating agent- induced DNA damage. *Ann Neurol* 21, 250-8 (1987)
40. Li JC & E Kaminkas: Deficient repair of DNA lesions in Alzheimer's disease fibroblasts. *Biochem Biophys Res Commun* 129, 733-8 (1985)
41. Bradley W , RJ Polinsky, WW Pendlebury, SK Jones, LE Nee, JD Bartlett, JN Hartshorn, R Tandan, L Sweet, GK Magin: DNA repair deficiency for alkylation damage in cells from Alzheimer's disease patients. *Prog Clin Biol Res* 317, 715-32 (1989)
42. Parshad RP, KK Sanford, FM Price, LK Melnick, LE Nee, MB Schapiro, RE Tarone & JH Robbins: Fluorescent light-induced chromatid breaks distinguish Alzheimer disease cells from normal cells in tissue culture. *Proc Natl Acad Sci U S A*, 93, 5146-50 (1996)
43. Trippi F, N Botto, R Scarpato, L Petrozzi, U Bonuccelli, S Latorraca, S Sorbi, & L Migliore: Spontaneous and induced chromosome damage in somatic cells of sporadic and familial Alzheimer's disease patients. *Mutagenesis* 16, 323-7 (2001)
44. Gasparini L, L Benussi, A Bianchetti, G Binetti, D Curti, S Govoni, S Moraschi, M Racchi, & M Trabucchi: Energy metabolism inhibition impairs amyloid precursor protein secretion from Alzheimer's fibroblasts. *Neurosci Lett* 263, 197-200 (1999)
45. Zhao WQ, P Manickam, O Zohar, G Chen, L Ravindranath, AS Mohamed, CG Lyketsos, R Zahorchak, R Etcheberrigaray & DL Alkon: Dysfunction of MAP kinase signaling in Alzheimer's Disease. *Soc Neurosci Abstr* 27, 354.13 (2001)

46. Guttman RP & GV Johnson: Oxidative stress inhibits calpain activity in situ. *J Biol Chem* 273, 13331-8 (1998)
47. Troncoso JC, A Costello, AL Watson Jr, & GV Johnson: In vitro polymerization of oxidized tau into filaments. *Brain Res* 613, 313-6 (1993)
48. Misonou H, M Morishima-Kawashima & Y Ihara: Oxidative stress induces intracellular accumulation of amyloid beta- protein (Abeta) in human neuroblastoma cells. *Biochemistry* 39, 6951-9 (2000)
49. Gabuzda D, J Busciglio, L B Chen, P Matsudaira, & BA Yankner: Inhibition of energy metabolism alters the processing of amyloid precursor protein and induces a potentially amyloidogenic derivative. *J Biol Chem* 269, 13623-8 (1994)

Key words. Oxidative stress, Metabolism, Calcium, fibroblasts, Alzheimer's disease, Cells, CSF, Lymphocytes, Platelets, Review

Send correspondence to: Dr Gary E. Gibson, Weill Medical College of Cornell University at Burke Medical Research Institute, 785 Mamaroneck Avenue, White Plains, NY 10605, Tel: 914-5597-2291, Fax 914-597-2757, E-mail: ggibson@med.cornell.edu