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Original Contribution

Do glutathione levels decline in aging human brain?

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ABSTRACT

For the past 60 years a major theory of "aging" is that age-related damage is largely caused by excessive uncompensated oxidative stress. The ubiquitous tripeptide glutathione is a major antioxidant defense mechanism against reactive free radicals and has also served as a marker of changes in oxidative stress. Some (albeit conflicting) animal data suggest a loss of glutathione in brain senescence, which might compromise the ability of the aging brain to meet the demands of oxidative stress. Our objective was to establish whether advancing age is associated with glutathione deficiency in human brain. We measured reduced glutathione (GSH) levels in multiple regions of autopsied brain of normal subjects ($n=74$) aged one day to 99 years. Brain GSH levels during the infancy/teenage years were generally similar to those in the oldest examined adult group (76–99 years). During adulthood (23–99 years) GSH levels remained either stable (occipital cortex) or increased (caudate nucleus, frontal and cerebellar cortices). To the extent that GSH levels represent glutathione antioxidant capacity, our postmortem data suggest that human brain aging is not associated with declining glutathione status. We suggest that aged healthy human brains can maintain antioxidant capacity related to glutathione and that an age-related increase in GSH levels in some brain regions might possibly be a compensatory response to increased oxidative stress. Since our findings, although suggestive, suffer from the generic limitations of all postmortem brain studies, we also suggest the need for "replication" investigations employing the new ^{1}H MRS imaging procedures in living human brain.

1. Introduction

Oxidative stress is considered a hallmark of human aging [1–4]; accordingly a variety of antioxidants have been avidly sought as possible dietary supplements or therapeutic reagents for the purpose of anti-aging and protection from degenerative aging changes [5,6]. The antioxidant glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH, the reduced form), which is synthesized de novo by γ-glutamyl cysteine ligase and glutathione synthetase, plays a pivotal role in supporting oxidative defense and maintaining redox equilibrium of the brain [7]. Glutathione participates in elimination of xenobiotics and hydrogen/organic peroxides through the actions of glutathione S-transferase and glutathione peroxidase, respectively, and can be recycled from the oxidized form (GSSG) to the reduced form (GSH) by glutathione reductase. Below normal levels of GSH have been reported in autopsied brain of patients with some neurodegenerative disorders [8–12] and it has been suggested that an age-related decline in glutathione in the normal human might "…contribute to many of the age-related declines in cellular function as well as the increased susceptibility to various insults" (see [13], p. 309). This glutathione-aging hypothesis has raised the interesting possibility that supplementation of GSH in the forms of its precursors, e.g., N-acetylcysteine, might be helpful in slowing down the progression of aging-related human disorders [14–16].

The cornerstone of the "GSH deficiency" hypothesis of brain

Abbreviations: GSH, Reduced glutathione; GSSG, Oxidized glutathione; MRS, Magnetic resonance spectroscopy; NSE, Neuron specific enolase; PMI, Postmortem interval

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aging is the preclinical observations of decreased levels of GSH in peripheral organs [17] and brain [18–20] of senescent as compared to mature animals. However, a review of the preclinical literature emphasized the lack of consensus in reports on aging- and development-related changes in brain levels of GSH, sometimes even in the same strain, sex, and the brain regions examined (see Section 4.2). There is also little information regarding changes in levels of GSH in human brain aging with the exception of a preliminary proton magnetic resonance spectroscopy (^{1}H MRS) report of decreased GSH levels in the occipital lobe of elderly *versus* young subjects [21] and a postmortem observation of decreased hippocampal GSH levels with aging in brain of a cohort of subjects suffering from head trauma ([22,23]; see Section 4.2).

Given the uncertain state of the literature on glutathione and brain aging in humans and the potential clinical therapeutic relevance of this question, the aim of our study was to establish whether levels of reduced and oxidized GSH change in developing and aging postmortem human brain. For this purpose, a representative number ($n=74$) of autopsied human brain from infancy to senescence was employed. Alterations in GSH levels across brain areas were explored for region-specific changes and we hypothesized, based on some, albeit conflicting, animal literature, that levels of GSH might decline with advancing age.

2. Materials and methods

This study was approved by the Centre for Addiction and Mental Health Research Ethics Board. Brain tissues were obtained at necropsy from a total of 74 subjects (male, $n=44$; female, $n=30$) who died without evidence of neurological or psychiatric disease (see Table 1 for subject information and known or suspected cause of death) or brain pathology when the fixed half brain was used for neuropathological examination [postmortem interval (PMI, from 3 to 27 h), 12.9 ± 0.8 h, mean $\pm$ SEM]. The agonal status of the subjects (Table 1) was classified according to their mode of death following the criteria of Hardy [24]: 1, violent fast death; 2, fast death of natural causes; 3, intermediate unexpected death; and 4, slow death with prolonged terminal phase. At necropsy, each brain was removed and divided midsagitally. One half of the brain (left, $n=43$; right, $n=31$) was frozen at -80 °C until dissection for biochemical analysis. The brain regions were dissected as previously described [25] and using the Atlas of Riley [26] for the caudate and Brodmann classification for cerebral cortical brain areas (frontal cortex, BA9; occipital cortex, BA17). Levels of GSH and GSSG were measured by a coulometric method using high performance liquid chromatography (HPLC; Spectrophysics SP8800 HPLC pump, ThermoFinnigan) and electrochemical detection (ESA Coulochem 2; ESA, Chelmsford, MA) with coulometric cells (ESA guard cell 5020 and analytical cell 5011) as previously described [12]. Briefly,

Table 1
Subject information[a].

Case#/sex/ side	Age[b]	PMI (h)	Agonal[c]	Probable cause of death	Case#/sex/ side	Age[b]	PMI (h)	Agonal[c]	Probable cause of death
1/F/L	21 h	20	3	Birth asphyxia	38/M/R	28	17	2	Pulmonary embolism
2/F/L	8 d	9	2	Hypoplastic left heart	39/F/L	28	7	1	Homicide hemorrhage
3/F/R	9 d	8	2	Hypoplastic left heart	*40/M/R	31	13	2	Cardiomyopathy
4/M/L	9 d	10	2	Pulmonary atresia	41/M/R	36	20.5	1	Multiple trauma accident
5/M/R	21 d	3	–	Unknown	42/M/R	37	10.3	2	Pulmonary embolism
6/F/L	24 d	23	4	Asphyxia	43/M/L	38	8.5	2	ASCVD
7/M/L	26 d	5	3	TAPVD	44/M/R	41	5	3	ASCVD
8/F/L	28 d	14	3	Gastroenteritis	45/M/R	44	13	2	ASCVD
9/M/L	35 d	3.5	2	Nesidioblastosis	46/M/R	47	23	2	ASCVD
10/M/R	2 m	18	2	CHF	#47/M/L	48	5.25	2	Cardiomyopathy
11/M	2 m	10	2	Cardiomyopathy	#48/F/R	48	22.3	2	ASCVD
12/F/L	3.5 m	9	2	Myocarditis	49/M/L	50	10.3	2	HASCVD
13/F/R	4 m	12	3	Sepsis	50/F/R	55	20.5	4	Bronchopneumonia
14/M/L	4.5 m	10	2	Primary hypertension	51/F/R	58	13	4	Pulmonary edema
15/M/R	6 m	6	2	Coarctation	52/F/L	59	21.5	2	HASCVD
16/M/L	6.5 m	16	3	Pneumonia	53/F/L	60	18.5	4	Bronchial hemorrhage
17/M/R	7 m	15	2	Myocarditis	54/F/L	66	3	2	Pulmonary embolism
18/M/L	7 m	26	–	Unknown	55/M/R	69	12	4	Renal failure/pneumonia
19/F/L	8 m	18	3	Gastroenteritis	56/F/R	70	9.5	2	MI, ASCAD
20/M/L	8 m	15	1	Acute trauma abdomen	57/M/R	71	15	2	MI, CHF
21/F/L	10 m	21	2	Drowning	58/M/R	71	17.5	2	Natural death
22/M/L	11 m	12	2	Drowning	59/M/R	72	8.5	2	MI
23/F/L	1.5	7	3	Pulmonary atresia	60/F/R	72	24.8	2	Cardiomyopathy
24/M/L	1.7	16	2	Post repair for TOF	61/M/L	73	16.3	2	MI
25/F/L	2	6	1	Abdominal trauma	62/F/R	73	7.5	4	Pulmonary consolidation, breast cancer
26/M/L	2	10	2	Unexpected death	63/M/R	76	4	3	Exsanguination
27/M/L	2.7	7	3	Small bowel obstruction	64/F/L	77	11	2	MI
28/M/R	3.5	11	1	Chest trauma	65/F/R	80	10	3	Atheroembolization
29/M/L	4	9	3	Chronic heart disease	66/F/R	80	10.5	2	MI
30/M/L	7	22	3	Acute adrenal insufficiency	67/M/R	80	15	2	HASCVD
31/F/L	11	17	2	Congenital heart disease	68/F/L	83	3	2	MI
32/M/R	13	27	1	Gunshot to heart	69/M/L	83	22	2	CHF
33/F/L	14	12	3	Renal failure	70/M/L	86	9.5	3	Hypertension, coronary disease, arrhythmia
34/M/R	17	6.5	1	Abdominal trauma	71/M/L	87	12	4	Diffuse interstial pulmonary disease
35/M/L	18	16.5	1	Multiple trauma accident	72/F/L	89	8	2	CHF
36/M/L	23	25	4	Morbid obesity	73/F/R	92	3.5	–	Unknown
#37/M/L	24	5.25	1	Multiple trauma accident	74/F/L	99	24	–	Unknown

[a] Subjects are Caucasians unless otherwise indicated (*Asian; #African American).
[b] Ages are in years unless otherwise indicated (h, hours; d, days; m, months).
[c] Agonal status of subjects was classified according to the criteria of Hardy et al. (1985) [24] [1, violent fast death; 2, natural fast death; 3, intermediate unexpected death; 4, slow death]. M, male; F, female; L, left side; R, right side; PMI, postmortem interval; ASCAD, atherosclerotic coronary artery disease; ASCVD, arteriosclerotic cardiovascular disease; CHF, congestive heart failure; HASCVD, hypertensive ASCVD; MI, myocardial infarction; TAPVD, total anomalous pulmonary venous drainage; TOF, Tetralogy of Fallot.