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Assessment of Anti-aging Efficacy of the Master Antioxidant Glutathione

Prashant Agarwal[a], Krishan Kant[b], Shubham Kaushik[c], Apoorv Soni[d], Lyndon Calisto Fernandes[e], Gowthami R[f], Ishita Mandal[g], Vaishnavi Padaki[h], Lalit Chetan Kandangkel[i], Ruchi khanna[j*]

[j]*Rass Biosolution Pvt Ltd, Kanpur,208001, India*

[j]*Email: ruchi@rass-biosolution.com*

Abstract

A chief tripeptide antioxidant Glutathione (GSH) is present inside each body cell which may have a profound effect in the control of aging. The anti-aging potency of GSH and its role towards the progression of certain age-related disease is still unclear. Glutathione based articles were searched on PubMEd database since the very first study of glutathione related to its discovery in 1923 to its present status till 2016**.** The data was made more informative and precise by searching glutathione relevant reports on google. Those articles were selected which were indicating the association of glutathione with the progression of age-related diseases, pre-clinical and clinical studies and age-longevity effect. It was analyzed that the increased oxidative stress (elevated GSSG/GSH ratio) is responsible for the incidence of age-related diseases and different organs failure. The glutathione redox ratio (GSSG/GSH) was found to be more pro-oxidizing with aging which plays a chief role for the generation of reactive oxygen species (ROS) and subsequently damages the macromolecular structures affecting the normal body mechanisms and functions. The clinical data has recommended that glutathione is a potent therapeutic agent for the control of age-related diseases and experimental analysis has confirmed its prominent effect in age-longevity. In future, more research is required for the development of new GSH delivery systems, evaluation of GSH therapeutic efficacy and the direct effect of GSH supplementation in age longevity. GSH combinational therapy and increasing GSH bioavailability is an another area which can give promising results.

***Keywords*:** Glutathione; anti-aging; GSSG; GSH; ROS; oxidative stress.

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* Corresponding author.

1. Introduction

The incidence of age-related disorders proceeding to the organ failures is the foremost cause of deaths worldwide. Mortality as an outcome of aging is gaining research attention day by day. The diseases becoming more prevalent with aging includes osteoarthritis (OA), cataract, cardiac disorders, CNS (Central Nervous System) disorders, chronic renal disorders and reproductive disorders. Mortality is due to the development of various age related diseases or reduced performance of various organs. The mechanism underlying for the development of different age-related disorders and an effective therapeutic agent to control aging is still unclear. In the large number of available studies, the anti-oxidant potency of GSH has been widely reported, but its anti-aging capability is still not highlighted.

Glutathione is actively synthesized by the animal cells and is present inside the cell at millimolar concentration. A remarkable decline in the intracellular antioxidant glutathione, most noticeably in brain, has been reported in several age-related diseases [1]. The aim of present review is to critically examine the evidences available for the prevalence of certain age-related diseases as an outcome of the harmful effects of increased glutathione related oxidative stress.

2. Materials and methods

Glutathione based articles were searched on PubMEd database using the keyword glutathione and reports were also searched on google. The present review has analyzed glutathione articles since the glutathione discovery in 1921 to its present status till 2016. The current review is compiled after screening out numerous glutathione study reports. Those reports were excluded that were suggesting the role of glutathione in skin whitening. The selection criteria included those articles that indicated alterations in glutathione level with ageing and the organ which is most susceptible to glutathione antioxidant dysregulation as a subject of aging. The anti aging data was made more precise by analyzing several pre-clinical experimental reports where in the effect of glutathione in age longevity was checked. Trend in the number of published research articles in successive decades were also compared using PubMed database.

3. Results

The analysis of glutathione articles using the key word glutathione on PubMed database searched a total of 1,29,680 articles. Those articles were screen out that were informative of the basic structure, properties and anti-aging effect of glutathione with an earliest report on the glutathione constitution in the year 1923. The findings of glutathione articles on google suggested that, in 1921, glutathione was first reported as the autoxidisable constituent present inside the cell. The current review is stressing on those reports that have found glutathione as a master anti-aging agent and the age longevity pre-clinical studies. The progression of age-related diseases with aging has been consistently reported to be the outcome of the dysregulation in glutathione antioxidant system. An analysis done for the number of glutathione research articles published per decade on PubMed database found an increasing trend which is further indicating that glutathione is gaining more and more of research attention with time (Table 1).

Table 1: Table indicating the increasing trend of number of research articles published in the respective decade since the beginning of glutathione research

Year range	Number of articles published
1928-1937	43
1938-1947	42
1948-1957	403
1958-1967	1,435
1968-1977	3,608
1978-1987	9,177
1988-1997	23,575
1998-2007	42,054
2008-2017	50,121

Discovery, sources, basic structure and properties of Glutathione

Glutathione was first identified as the autoxidisable constituent in the yeast cell and many animal tissues. It was initially analyzed as a dipeptide of glutamic acid and cysteine and found intracellular at a low concentration (0.01-0.02%) and its cysteine moiety is responsible for the reversible changes in sulfur group from sulphydryl to the disulphide condition [2]. A reinvestigation study revealed it to be a tri-peptide of glycine, glutamic acid and cysteine [3]. The common name of low molecular weight water-soluble thiol-tripeptide glutathione is γ-L-Glutamyl-L-cysteinylglycine having chemical formula and molar mass of $C_{10}H_{17}N_3O_6S$ and 307.32 $g \cdot mol^{-1}$respectively.

Modifications in glutathione redox state (increased oxidized glutathione (GSSG)/ GSH) as an outcome of aging

A shift in the cellular glutathione redox state {reduced glutathione (GSH): oxidized glutathione (GSSG) ratio} towards more pro-oxidizing with aging has been reported in several studies. The GSSG content in mice has been found to be significantly increased to 84% with age, whereas the GSH content remained intact hence concluding a major alteration in glutathione redox state with aging [4].

Experimental Study attributing the role of GSH in age expansion

The experimental data analyzed that on GCL over expression, the mean and maximum life spans of drosophila were increased with 50% [5]. This study significantly confirmed the age longevity effect of glutathione.

Studies on the deleterious mechanisms with the age-related alteration in the glutathione

Glutathione has reported to play a key role in defending the cell against the oxidative stress thereby acting as a

free radical scavenger. As compared with the young ones, the older persons have been analyzed with an increased oxidative stress as supported by a large number of studies. The protection of cell against ROS, such as, H_2O_2, hypochlorous acid (HClO), free radical superoxide ($O2^{-2}$) and hydroxyl radical (-OH), is strongly hindered by the alteration in glutathione antioxidant system with aging, which further reduces the ability to prevent the progression of certain age-related diseases. The development of hydroxyl free radical is increased due to the variation in GSH antioxidant system which is responsible for the enhanced lipid peroxidation and rise in the level of products like, malondialdehyde and hydroxynonenals. These products form adducts with nucleic acids or proteins and their structure and functions get modified [6, 7]. Another consequence of GSH imbalance is the development of protein mixed disulfides which is responsible for the obstruction of catalytic efficiency of enzymes and thus hindering the adaptive responses stressful conditions [8, 9].

Evidences of age-related low GSH content (increased oxidative stress) leading to the progression of certain diseases prevalent with aging

Age-related low GSH content leading to poor chondrocyte survival

It has been reported that the human chondrocyte cells of younger donors survived significantly better than chondrocytes derived from older donors in addition to low GSSG/ GSH ratio in the cells of young donor, whereas the level of GSH indicated no significant difference in the cells of both the ages [10]. The extracellular matrix (ECM) is also reported to be adversely affected along with the reduced synthesis of proteoglycan and hyaluronic acid of articular cartilage due to the enhanced ROS production resulted with the reduced antioxidant capacity of the glutathione system. These only are the changes to be responsible for the development of osteoarthritis (OA), which becomes much more prevalent after the age of 50 [11, 12].

Age-related low glutathione content leading to cataract

The concentration of the chief antioxidant molecule GSH in the eye lens is 2–4 mM and is required for the maintenance of clear lenses [13, 14]. The content of glutathione in mammalian eye is high which turns out to be low in cataract leading to immense suffering of the patient. The reduced synthesis and recycling of GSH and an elevated GSSG level is mainly investigated as the result of age-related fall in glutathione synthesis enzymes and glutathione reductase (GR) activity [15-19]. This is further responsible for the formation of human subcapsular cataract by making the cells more susceptible to oxidative damage by peroxide [20]. It has been observed that *in vitro* the single-strand breaks in chromosomal telomeres of human retinal pigment epithelial cells are induced by the oxidative stress [21]. Similarly, in a GSH-deficient transgenic mice the DNA strand breaks were developed on exposure to H_2O_2 [22, 23]. Such an amplified oxidative stress due to reduced GSH is the key initiating factor in the development of age-related cataracts which acts as a prominent cause of blindness worldwide.

Age-related low glutathione content leading to heart diseases

The age-related disturbances in glutathione antioxidant system are found to be capable of damaging the human heart and further leading to heart failure. In human atherosclerotic lesions, a weak glutathione-related enzymatic antioxidant shield is present which is affected with glutathione antioxidant system imbalance in vascular wall in

human atherogenic processes [24]. It is also evidenced that there is a remarkable reduction in blood glutathione level before rise in TNFR1 (marker of symptomatic heart failure severity) in the patients of different heart diseases.

This investigation recommended that blood glutathione test as a new biomarker to detect cardiac abnormalities cardiac abnormalities [25].

Age-related low glutathione content leading to Central Nervous System damage

The variation in the glutathione antioxidant system is reported to be capable of causing the improper functioning of brain turning with the progression of diseases, such as, Alzheimer`s disease (AD), Parkinson`s disease (PD), Schizophrenia. Glutathione related oxidative stress has been found to play a prominent role in the development of brain diseases by dysfunction of protein, lipid, mitochondria, neurons; nucleic acid oxidation, impaired autophagy and further cell death. The chief reason behind the GSH deficiency in brain is the low level of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activity [26]. It is notably analyzed that PD is most primarily the outcome of glutathione depletion [27, 28]. In brain, the GSH loss is strongest and corresponded to the development of PD and neuronal injury following stroke [1].

Age-related low glutathione content leading to chronic renal failure

The chronic renal failure (CRF) patients have consistently been reported with an oxidative stress as compared to healthy controls in several studies [29, 30] with a lower glutathione levels and reduced enzymatic activities of glutathione peroxidase and glutathione reductase [31].

Age-related low glutathione content leading to infertility

The leading cause of infertility in the population of both men and women is the reproductive aging associated with reduced intracellular GSH level. On comparing with control the GSH concentrations in ollgozoospermla patients were significantly lower and causing male infertility [32, 33]. The deleterious effects of increased oxidative stress with aging involved affected cytoskeletal fibres, fertilization and embryo development [34]. Glutathione is crucial for the antioxidant defenses of spermatogenic epithelium, the epididymis and ejaculated spermatozoa [35]. Glutathione has been found to exhibit significant therapeutic effect on dyspermia associated with some andrological pathologies causing male infertility [36].

In addition, female infertility involving the cessation of ovarian follicular activity and poor pregnancy outcome is also found to be an outcome of the oxidative stress. The perimenopausal and postmenopausal phases as compared to the reproductive phase have been found to be associated with reduced GSH levels and enzymatic activities of GSH-Px, and SOD [37]. The hormonal therapy provided to post-menopausal women reduced the level of oxidative stress [38, 39].

The data available has led to the clear conclusion that reduced GSH level is the leading cause of infertility or reproductive aging in both the genders. GSH decrease in men is responsible for the diseases associated with

male infertility and in female responsible for the hormonal disorders.

Clinical studies proving the therapeutic role of glutathione

Glutathione has been clinically proven as an effective therapeutic agent in a large number of studies. The progression of Parkinson`s disease is effectively retarded by the intravenous administration of GSH [40]. In non—insulin-dependent diabetes mellitus patients, the total glucose uptake and both intra erythrocytic GSH/GSSG ratio were significantly increased by intravenous GSH infusion [41]. The patients of dyspermia were also positively cured by GSH [42]. Various skin inflammatory diseases like psoriasis have been recommended with the treatment of GSH [43]. In addition, TER286 and TER199 are the two glutathione based compounds acting as a pro-drug for cancer chemotherapy [44, 45].

4. Discussion

The present systematic review is highlighting glutathione as a chief anti-aging agent. A low content of glutathione is responsible for the progression of certain age-related diseases associated with heart, eye, reproductive, CNS, kidney, cartilage etc. leading to the organ failure and subsequent death. In view of such an efficient anti-oxidant, and thus, anti-aging capability of GSH, there is a requirement of new approaches of increasing the cellular GSH level for the prevention of certain age-related diseases. In this direction, more research is required for the development of new methods of external GSH delivery system so as to increase its bioavailability or by helping human cells to synthesize more GSH. Further studies on GSH combinational therapy are required to be carried out for an enhanced effect of different available approaches to prevent different diseases. In addition to this, new clinical studies are required to be carried out for understanding the clear role of GSH in preventing the progression of disease. Further, more clinical investigations are required pertaining to the direct effect of GSH supplementation in age longevity. Several clinical studies are also required to investigate the effect of GSH supplementation in immuno compromised patients and in slowing down the progression of several diseases for which proper treatment is not available.

5. Conflict of Interest

None of the authors had a personal or financial conflict of interest.

References

[1]. Pamela M. The effects of stress and aging on glutathione metabolism.Ageing Res Rev 2005;4:2288–314.

[2]. Hopkins FG. On an autoxidisable constituent of the cell. Biochemical Journal. 1921;15(2):286.

[3]. Hopkins FG. On glutathione: a reinvestigation. Journal of Biological Chemistry. 1929 Oct 1;84(1):269-320.

[4]. Toroser D, Yarian CS, Orr WC, Sohal RS. Mechanisms of gamma-glutamylcysteine ligase regulation. BiochimBiophysActa 2006;1760:233–244.

[5]. Ohal RS. Overexpression of glutamate-cysteine ligase extends life span in Drosophila melanogaster. J

BiolChem 2005;280:37331–37338.

[6]. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free RadicBiol Med 1991;11:81–128.

[7]. Uchida K, Stadtman ER. Quantitation of 4-hydroxynonenal protein adducts. Methods MolBiol 2000;99:25–34.

[8]. Cotgreave IA, Gerdes RG. Recent trends in glutathione biochemistry-glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation? BiochemBiophys Res Commun 1998;242:1–9.

[9]. Orr WC, Radyuk SN, Prabhudesai L, Toroser D, Benes JJ, Luchak JM, Mockett RJ, Rebrin I, Hubbard JG, Sohal RS. Overexpression of glutamate-cysteine ligase extends life span in Drosophila melanogaster. J BiolChem 2005;280:37331–8.

[10]. Carlo MD, Loeser RF. Increased Oxidative Stress with aging reduces chondrocyte survival correlation with intracellular glutathione levels. Arthritis Rheum 2003;48: 3419–30.

[11]. Baker MS, Feigan J, Lowther DA. Chondrocyte antioxidant defences: the roles of catalase and glutathione peroxidase in protection against H_2O_2 dependent inhibition of proteoglycan biosynthesis. J Rheumatol 1988;15:670–77.

[12]. Loeser RF Jr. Aging and the etiopathogenesis and treatment of osteoarthritis. Rheum Dis Clin North Am 2000;26:547–67.

[13]. Giblin FJ. Glutathione: a vital lens antioxidant. J OculPharmacolTher 2000;16:121 –135.

[14]. MichaelR, Bron AJ. The ageing lens and cataract: a model of normal and pathological ageing. Philos Trans R SocLond B BiolSci 2011;366:1278–1292.

[15]. Zhang WZ, Augusteyn RC. Ageing of glutathione reductase in the lens. Exp Eye Res 1994;59:91–95.

[16]. Xing KY, Lou MF. Effect of age on the thioltransferase (glutaredoxin) and thioredoxin systems in the human lens. Invest Ophthalmol Vis Sci 2010;51:6598–6604.

[17]. Zhu Y, Carvey PM, Ling Z. Age-related changes in glutathione and glutathione-related enzymes in rat brain. Brain Res 2006;1090:35–44.

[18]. Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL. Glutathione dysregulation and the etiology and progression of human diseases. BiolChem 2009;390:191–214.

[19]. Spector A. Oxidative stress-induced cataract: mechanism of action. FASEB J 1995; 9:1173–1182.

[20]. Rathbun WB, Schmidt AJ, Holleschau AM. Activity loss of glutathione synthesis enzymes associated with human subcapsular cataract. Invest Ophthalmol Vis Sci1993;34:2049-54.

[21]. Shigeru H, Leonard M, Hjelmeland, Handa JT. Oxidative Stress–Induced single-strand breaks in chromosomal telomeres of human retinal pigment epithelial cells in vitro. Invest Ophthalmol Vis Sci 2001;42:2139-44.

[22]. Kleiman NJ, Spector A. DNA single strand breaks in human lens epithelial cells from patients with cataract. Curr Eye Res 1993;12:423–431.

[23]. Reddy VN, Lin LR, Ho YS, Magnenat JL, Ibaraki N, Giblin FJ, Dang L. Peroxideinduced damage in lenses of transgenic mice with deficient and elevated levels of glutathione peroxidase. Ophthalmologica 1997;211:192 –200.

[24]. Lapenna D, Gioia S, Ciofani G, Mezzetti A, Ucchino S, Calafiore AM, Napolitano AM, Ilio CD,

Cuccurullo F. Glutathione-related antioxidant defenses in humanatherosclerotic plaques. Circulation 1998;97:1930-1934.

[25]. Damy T, Kirsch M, Khouzami L, Caramelle P, Corvoisier PL, Thoraval FR, Dubois-Rande J, Hittinger L, Pavoine C, Pecker F. Glutathione deficiency in cardiac patients is related to the functional status and structural cardiac abnormalities. PLoSOne 2009 Mar 25 (Epub ahead of print; DOI: doi: 10.1371/journal.pone.0004871.)

[26]. Padurariu M, Ciobica A, Hritcu L, Stoica B, Bild W, Stefanescu C. Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer's disease. NeurosciLett 2010;469:6–10.

[27]. Sian, J. Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F, Jenner P, Marsden CD.Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann Neurol 1994;36:348–55.

[28]. Sofic E, Lange KW, Jellinger K, Riederer P. Reduced and oxidized glutathione in the substantianigra of patients with Parkinson's disease. NeurosciLett 1992;142:128–130.

[29]. Annuk M , Fellstom B, Akerblom O, Zilmer K, Vihalemm T, Zilmer M. Oxidative stress markers in pre-uremic patients. ClinNephrol 2001; 56:308-314.

[30]. Annuk M, Zilmer M, Lind L, Linde T, Fellstrom B. Oxidative Stress and Endothelial Function in Chronic Renal Failure. J Am SocNephrol 2001;12:2747-52.

[31]. Martin-Mateo MC, Sanchez-Portugal M, Iglesias S, Paula A, Bustamante J. Clinical Study: Oxidative Stress in Chronic Renal Failure. Ren Fail2009;21:155-167.

[32]. Mimic-Oka J, Ekmescic V, Dragicevic P.Erythrocyte glutathione peroxidase and superoxide dismutase activities in different stages of chronic renal failure.ClinNephrol 1995;44:44-48.

[33]. Ochseedorf FR,Buhl R, Bastlein A, Beschmann H. Glutathione le spermatozoa and seminal plasma of infertile men. Hum Reprod1998;13:353-359.

[34]. Tarín JJ.Potential effects of age-associated oxidative stress on mammalian oocytes/embryos. Mol Hum Reprod1996;2:717-724.

[35]. IrvineDS.Glutathione as a treatment for male infertility. The journal of the society for reproductive and fertility.Rev Reprod 1996;1:6-12.

[36]. Lenzi A, Lombardo F, Gandini L, Culasso F, Dondero F.Glutathione therapy for male infertility. Arch Androl1992;29:65-8.

[37]. Ogunro PS, Bolarinde AA, Owa OO, Salawu AA, Oshodi AA. Antioxidant status and reproductive hormones in women during reproductive, perimenopausal and postmenopausal phase of life.Afr J Med MedSci2014;43(1):49-57.

[38]. Mueck AO, Seeger H. Estrogens acting as cardiovascular agents: Direct vascular actions. Curr Med ChemCardiovascHemtaol Agents 2004;2:35–42.

[39]. Suleyman D, Yilmaz M, Koseogul M, Akalini N, Aslani D, Aydin A. Role of free radicals in peptic ulcer and gastritis. Turk J Gastroenterol 2003;14: 39-43.

[40]. SechiG, Deledda MG, Bua G, Satta WM, Deiana GA, PesGM, RosatiG. Reduced intravenous glutathione in the treatment of early parkinson's disease. ProgNeuro-PsychophBiolPsychiatry 1996;20: 1159–1170.

[41]. Mattia G, Bravi MC, Laurenti O, Cassone-Faldetta M, Armiento A, Ferri C, Balsano F. Influence of reduced glutathione infusion on glucose metabolism in patients with non-insulin-dependent diabetes mellitus.Metabolism1998;47:993-7.

[42]. Annuk M , Fellstom B, Akerblom O, Zilmer K, Vihalemm T, Zilmer M. Oxidative stress markers in pre-uremic patients. ClinNephrol 2001; 56:308-314.

[43]. Jurima-Romet M, Shek PN. Lung uptake of liposome-entrapped glutathione after intratracheal administration. J Pharm Pharmacol 1991;43:6-10.

[44]. Kauvar LM, Morgan AS, Sanderson PE, Henner WD. Glutathione based approaches to improving cancer treatment.ChemBiol Interact 1998;111-112:225-38.

[45]. Rosario LA, Brien ML, Henderson CJ, Wolf CR, Tew KD. Cellular response to a glutathione S-Transferase P1-1 activated prodrug. MolPharmacol 2000;58:167–174.