ORIGINAL ARTICLE

Age-Dependent Changes in Glutathione-S-Transferase: Correlation with Total Plasma Antioxidant Potential and Red Cell Intracellular Glutathione

Pawan Kumar Maurya · Syed Ibrahim Rizvi

Abstract The correlation between antioxidant capacity and oxidative damage during aging has been reported in several tissues in different species. Glutathione-S-transferases (GST) can metabolise endogenous and exogenous toxins and carcinogens by catalysing the conjugation of diverse electrophiles with reduced glutathione (GSH). We observe a significant ($P < 0.001$) increase in plasma GST activity as a function of human age (r = 0.5675). A significant ($P < 0.001$) positive correlation (r = 0.8979) is observed between GST activity and total plasma antioxidant potential measured as ferric reducing ability of the plasma (FRAP). GST activity and red cell intracellular GSH also show a significant positive correlation (r = 0.7014). We hypothesize that the increased activity of plasma GST is a manifestation of increased generation of ROS and a concomitant decrease in the level of plasma antioxidant capacity during aging.

Keywords Glutathione-S-transferase · Human aging · Oxidative stress

Introduction

Oxidative stress results when production of reactive oxidative species (ROS) exceeds the capacity of cellular antioxidant defenses to remove these toxic species [1, 2].

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The correlation between antioxidant capacity and oxidative damage during aging has been reported in several tissues in different species [3–5], however data on changes of oxidative stress markers in plasma and erythrocytes of healthy populations during aging are few and sometimes contradictory [6–8]. Recently we have reported a significant age dependent decline in plasma antioxidant capacity, measured in terms of Ferric Reducing Ability of the Plasma (FRAP) values [9]. Since decrease in oxidative damage is related to dietary intake of antioxidants [10], it is important to study the correlation between antioxidant capacity of the plasma and markers of oxidative stress in different populations.

The glutathione-S-transferases (GST) can metabolise endogenous and exogenous toxins and carcinogens by catalysing the conjugation of diverse electrophiles with reduced glutathione (GSH). In the present study we report the age-dependent alteration in the activity of plasma GST in Indian population, we also correlate this parameter with total plasma antioxidant potential measured in terms of FRAP values [9] and with red cell reduced glutathione (GSH) which has already been reported to decrease as a function of human age [11].

Materials and Methods

The study was carried out on 80 normal healthy subjects of both sexes between the ages of 18–85 years. The subjects were screened for diabetes mellitus, asthma, tuberculosis or any other major illness. None of the subjects were smokers or were taking any medication. All persons gave their informed consent for the use of their blood samples for the study. The protocol of study was in conformity with the guidelines of the Institutional Ethical Committee.

Human venous blood from different healthy volunteers was obtained by venipuncture in heparin. The blood was centrifuged at $1,800 \times g$ for 10 min at 4°C. After removal of plasma, buffy coat and upper 15% of the packed red blood cells, the RBC were washed twice with cold PBS (0.9% NaCl, 10 mM Na_2HPO_4, pH 7.4).

GST activity in plasma was assayed using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate [12]. The Ferric Reducing Ability of Plasma (FRAP) values were determined following the method of Benzie and Strain [13]. Erythrocyte MDA was measured according to the method of Esterbauer and Cheeseman [14].

Plasma protein was estimated following the method of Lowry et al. [15].

Statistical analyses were performed using the software PRISM 4. Relationships between various parameters were assessed using Pearson correlation coefficient (r).

Results and Discussion

Glutathione-S-transferases (GSTs) are crucial enzymes in the cell detoxification process catalyzing the nucleophilic attack of glutathione (GSH) on toxic electrophilic substrates and producing a less dangerous compounds. Studies on GSTs are of great importance since they have been implicated in the development of drug resistance in tumoral cells and are related to human age related diseases such as Parkinson's, Alzheimer's, atherosclerosis, liver cirrhosis, aging and cataract formation [16, 17]. We have previously reported increased activity of superoxide dismutase (SOD) and catalase (CAT) and decrease in GSH as a function of human age which have been explained on the basis of increased oxidative stress in higher age groups [11, 18].

We observe a significant ($P < 0.001$) positive correlation (r = 0.5675) between the activity of plasma glutathione-S-transferase (GST) and human age (Fig. 1). As already reported earlier, we observed a decrease in the antioxidant capacity of plasma measured in terms of FRAP values and a decrease in erythrocyte intracellular GSH as a function of human age [9, 11]. To analyse the correlation of plasma GST activity with plasma antioxidant capacity, we plot a quotient: GST activity/FRAP as a function of human age (Fig. 2). The plot shows a strong positive correlation (r = 0.8979) between GST activity and total plasma antioxidant potential. We also report strong positive correlation (r = 0.7014) between GST and red cell reduced glutathione by plotting quotient of GST/GSH as a function of human age (Fig. 3). Our results thus confirm that human aging is associated with increase in the activity of GST which correlates with decrease in antioxidant potential and GSH. The increased activity of GST could be due to a compensatory mechanism in response to increased
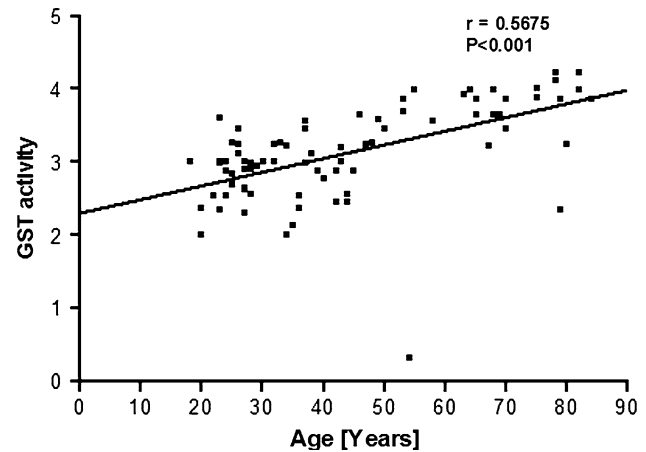
Fig. 1 Glutathione-S-transferase (GST) activity plotted as a function of human age. GST activity is expressed as U/mg protein
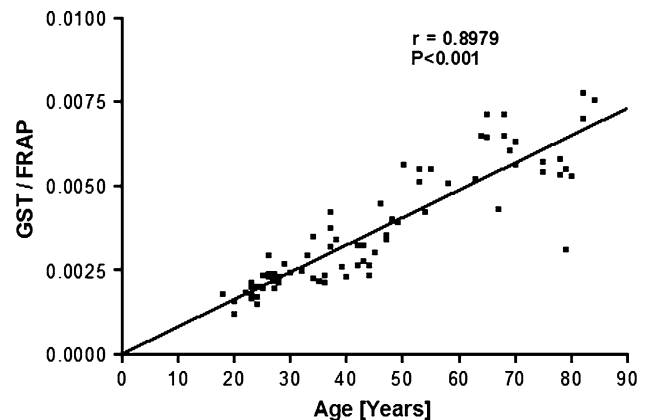
Fig. 2 Plot of quotient (GST/FRAP values) as a function of human age. FRAP (Ferric Reducing Ability of the Plasma) values expressed as μmol Fe(II) per liter of plasma. GST activity is expressed as U/mg protein
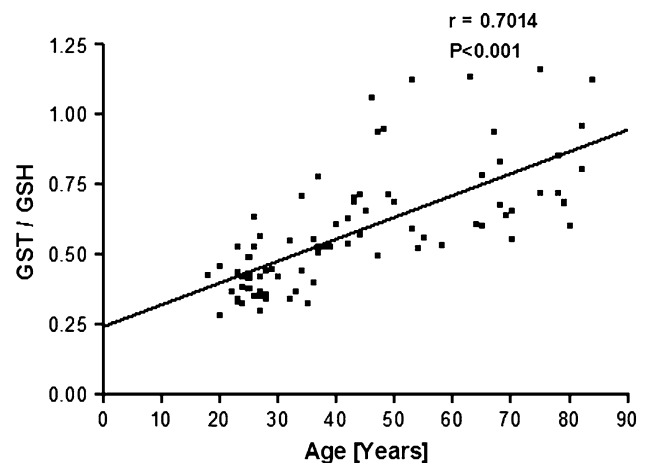
Fig. 3 Plot of quotient (GST/GSH values) as a function of human age. GSH is expressed as mg/ml PRBC (packed red blood cells). GST activity is expressed as U/mg protein

oxidative stress. The increased activity of GST is a manifestation of increased generation of ROS as a function of age in humans.

GST shows many important functions in mammals including detoxification, catalysis, metabolic associated functions, drug resistance relation and inhibition and in age related diseases [19, 20]. It has been reported that GST activity increase in conditions of stress [21]. Viewed in conjunction with our previous findings of age-dependent increase in activity of plasma SOD and CAT, and decreased antioxidant potential and GSH, we hypothesize that the human body has inherent compensatory mechanisms against oxidative stress, however, this capacity gets overwhelmed during aging. Our findings also emphasize the need to establish age-dependent reference values for GST involving their role in age related and different disease conditions.

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