

# Beta-amyloidolysis and glutathione in Alzheimer's disease

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**Abstract:** In this review, we hypothesized the importance of the interaction between the brain glutathione (GSH) system, the proteolytic tissue plasminogen activator (t-PA)/plasminogen/plasmin system, regulated by plasminogen activator inhibitor (PAI-1), and neuroserpin in the pathogenesis of Alzheimer's disease. The histopathological characteristic hallmark that gives personality to the diagnosis of Alzheimer's disease is the accumulation of neurofibril tangles located intracellularly in the brain, such as the protein tau and extracellular senile plaques made primarily of amyloid substance. These formations of complex etiology are intimately related to GSH, brain protective antioxidants, and the proteolytic system, in which t-PA plays a key role. There is scientific evidence that suggests a relationship between aging, a number of neurodegenerative disorders, and the excessive production of reactive oxygen species and accompanying decreased brain proteolysis. The plasminogen system in the brain is an essential proteolytic mechanism that effectively degrades amyloid peptides ("beta-amyloidolysis") through action of the plasmin, and this physiologic process may be considered to be a means of prevention of neurodegenerative disorders. In parallel to the decrease in GSH levels seen in aging, there is also a decrease in plasmin brain activity and a progressive decrease of t-PA activity, caused by a decrease in the expression of the t-PA together with an increase of the PAI-1 levels, which rise to an increment in the production of amyloid peptides and a lesser clearance of them. Better knowledge of the GSH mechanism and cerebral proteolysis will allow us to hypothesize about therapeutic practices.

**Keywords:** glutathione, Alzheimer's disease, t-PA, PAI-1, plasminogen

## Introduction

Sporadic or common Alzheimer's disease (AD) is a chronic process of complex etiology, without any existing effective treatment, with aging being the main etiological factor of universal risk.

The histopathological characteristic that gives personality to the diagnosis of AD is the accumulation of neurofibril tangles located intracellularly in the brain, such as protein tau<sup>1-6</sup> and senile plaques with extracellular amyloid beta (Ab) substance.<sup>7-13</sup> These deposits are produced as a consequence of a biological disorder in their production and elimination/clearance from the brain.<sup>10,13-17</sup> These formations of complex etiology are intimately related to glutathione (GSH), brain protective antioxidants, and proteolysis, in which tissue plasminogen activator (t-PA) plays a key role.<sup>18,19</sup>

Our review suggests that GSH may play an essential role in the physiopathology of the different components of the proteolytic mechanisms in the brain, and focuses on its relation to t-PA and the plasminogen/plasmin system.

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## Brain GSH

GSH (L-gamma-glutamyl-L-cysteinylglycine) is a tripeptide present in large quantities in all mammal cells and in small amounts extracellularly,<sup>20–22</sup> being mainly located in the cytosol, mitochondria, and endoplasmic reticulum.<sup>23</sup> It plays a very important role in many biological processes involved in organism homeostasis, most notably, in neutralizing the free radicals that produce reactive oxygen species (ROS) (due to its great antioxidant activity),<sup>20,24–28</sup> since oxidation is a basic process in the genesis of neurodegenerative disorders.<sup>29</sup>

GSH is the most important component of the antioxidant mechanism of the brain.<sup>30</sup> It has a relatively homogeneous distribution in rat brains,<sup>31–33</sup> reaching its highest levels in the brain cortex, corpus striatum, and the glia<sup>31,34</sup> but it diminishes significantly with aging.<sup>32,34,35</sup> GSH levels in neuronal cells are lower than in glia cells.<sup>36–38</sup> This difference can be due to a smaller reserve of precursors for the GSH synthesis, especially of cysteine.

In studies of brain cells, the concentration of GSH in different neuronal cells has been found to vary considerably.<sup>39,40</sup> Likewise, the GSH concentration in astroglia cell cultures has also been found to vary widely.<sup>39,41,42</sup> Other studies have shown lower GSH concentration levels in neurons than in astrocytes,<sup>39,43</sup> with the concentration of endogenous neuronal GSH ten times lower than in astrocytes.<sup>44</sup> The separation of neurons and glia cells in cocultures and then their later culture has shown a significantly decreased level of neuronal GSH whereas this remained constant in astroglia cells.<sup>43</sup> This difference in GSH levels between astrocytes and neurons was observed in the cortex; nevertheless, the concentrations are very similar in other parts of the brain, like the midbrain and the striatum.<sup>45</sup> It is possible that this presence and distribution in the brain is due to cellular specialization that confers a great capacity to generate free radicals, as a consequence of being an organ with high oxygen requirement (20% of the total consumption of the organism, in spite of being less than 2% of the body weight in human adults).<sup>46</sup>

Astrocytes protect neurons against the toxicity of free radicals by increasing their GSH levels, by means of the transfer of sulfated amino acids or peptides as precursors (mainly cysteine and the dipeptide CysGly).<sup>30,44,47–49</sup> An increase in cellular GSH concentration makes the neurons more resistant to cytotoxic injuries.<sup>50–52</sup>

Many cerebral functions are altered as a consequence of decrease in intra- and extracellular levels of GSH.<sup>53</sup> This decrease can be due to either the inhibition of its synthesis or its consumption in the conjugation with exogenous compounds.<sup>24,54</sup> The progressive decrease of GSH levels

resulting from aging and related illnesses, is of great interest for investigators.<sup>55,56</sup> The decrease of GSH levels has been detected not only in humans, but also in lesser animals, such as rodents and insects.<sup>57</sup> The decrease found with aging is linked to an increase of ROS.<sup>58</sup> There is scientific evidence of a relationship between aging and a number of neurodegenerative processes due to the excessive production of free radicals and the imbalance between the oxidant species and antioxidant defenses.<sup>59–61</sup>

Buthionine sulfoximine (S-(n-butyl) homocysteine sulfoximine) (BSO), a selective and potent inhibitor of the gamma-glutamylcysteine synthetase,<sup>24,62</sup> has been administered in previous investigations. BSO inhibits GSH biosynthesis and causes depletion of cellular GSH levels.<sup>21,22</sup> In rats, the same GSH decrease has also been achieved with the administration of diethyl maleate – the diethyl maleate reacts with GSH, causing the formation of conjugated GSH, which is then excreted.<sup>31,62–64</sup>

The effect of pharmacological depletion of cerebral GSH following exposure to BSO and diethyl maleate, in cellular cultures or after their administration to animals, has been studied over the last 20 years. From an experimental point of view, the administration of BSO by systemic route to adults has not been very effective in producing GSH depletion in the brain,<sup>65</sup> being effective only in newborn rats or mice, where it was facilitated by the immaturity of the blood–brain barrier (BBB).<sup>66</sup> Low levels in adult animals have been achieved using a direct intracerebroventricular<sup>65,67</sup> administration of BSO by means of stereotaxic technique<sup>68</sup> or by intrathecal administration.<sup>69</sup>

The decrease of GSH levels in the brain of newborn rats has been shown to frequently lead the animal to a fatal situation, as a consequence of the accumulation of hydrogen peroxide and subsequent mitochondrial lesion, thus showing the great metabolic importance of GSH.<sup>66,70</sup> Decrease in the number of cerebral mitochondria has also been observed<sup>71</sup> as well as the reduction in enzymatic activity of GSH reductase.<sup>72</sup> In investigations of cerebral ischemia, it has been observed that GSH depletion exacerbates cortical infarction and edemas after ischemia, due to an increasing presence of ROS.<sup>73</sup> In cultures of mesencephalic cells, incubation with BSO has caused a significant reduction of GSH, resulting in a loss of the integrity of the membrane and cellular death,<sup>74,75</sup> after the loss of mitochondrial GSH.<sup>76</sup> The depletion of cerebral GSH has also been found to modify the interaction between astrocytes and neurons, diminishing the neuronal protection against oxidant agents.<sup>44,77</sup>

Given the GSH decrease that is progressively produced with aging,<sup>57,60,78,79</sup> we cannot discard the hypothesis that

a defect in the mechanism of antioxidant cellular defense can be the silent trigger of the neurodegenerative process and neuronal death.<sup>72</sup> It may be that an imbalance in the equilibrium between the formation of free radicals and their neutralization (oxidation–reduction) leads to a situation of oxidative stress with great organic risk that can be associated with neurodegenerative illnesses,<sup>73,79,80</sup> including, most often, Parkinson's disease<sup>25</sup> and AD.<sup>33,74</sup>

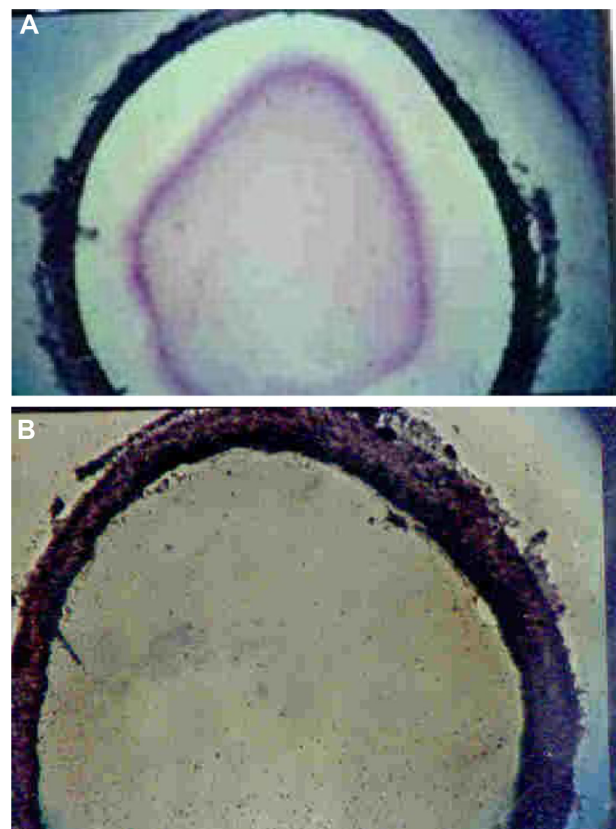
## Fibrinolytic activity and GSH

In 1959, Todd<sup>81</sup> devised a histochemical fibrin slide technique with which he demonstrated the existence of areas of lysis in vascular walls that were related to the presence of the activators of fibrinolysis. Using immunohistochemical methods, it was revealed that t-PA was present in the blood vessels of most organs and that it was synthesized by endothelial cells.<sup>82,83</sup>

More than 20 years ago<sup>62</sup> it was reported that the pharmacological effect of administration of BSO or diethyl maleate in rabbits is a significant decrease in GSH levels, accompanied by the inhibition of fibrinolytic activity important for the fibrin plate. This inhibition of plasmin activity results from a decrease in the liberation of t-PA at the cellular level and a significant increase in its inhibitor, plasminogen activator inhibitor (PAI-1), and occurs without any modification in the normal values of alpha-2 antiplasmin. These results lead us to consider that GSH could play an essential role in regulation of the different components of the fibrinolytic system.

Following an administration of BSO, to rabbits at a dose of 4.5 mmol/kg body weight liver, it was seen that GSH concentrations were reduced, with the greatest decrease (51%) occurring 7 hours after administration and with values that remained lowered after 24 hours; however, at 3 days posttreatment, GSH concentrations did not significantly differ from those in the control groups. Treatment with diethyl maleate, at a dose of 3.2 mmol/kg body weight, also induced a significant reduction in hepatic GSH levels that were 54% lower than those of the controls, after 45 minutes. GSH concentrations in the aortic arch were equally reduced ( $0.24 \pm 0.05 \mu\text{mol/g}$  liver and  $0.20 \pm 0.04 \mu\text{mol/g}$  liver) 7 hours after BSO or 45 minutes after diethyl maleate were administered, respectively; concentration in the control group was  $0.33 \pm 0.04 \mu\text{mol/g}$  liver).<sup>62</sup>

A study of the fibrinolytic activity in the aortic arch revealed an extensive area of lysis in the endothelial wall in the control rabbit groups.<sup>62</sup> Following an administration of BSO, fibrinolysis was inhibited (Figure 1A and B) and only reappeared 3 days later. Intraperitoneal injection of



**Figure 1** Inhibition of fibrinolytic activity by BSO, on arterial walls in rabbits. Photomicrographs of 30  $\mu\text{m}$  cross sections of the aortic arch that were incubated with fibrinogen, plasminogen, and human thrombin and stained with Harris' hematoxylin ( $\times 40$ ). **(A)** Fibrinolytic activity of the aortic arch reveals an extensive area of lysis under the endothelial wall. **(B)** Seven hours after BSO administration, the inhibited fibrinolysis activity could be attributed to a lower release of t-PA. **Note:** Plasma t-PA levels were decreased following administration of BSO and only reappeared 3 days later, when the level of plasma glutathione was also normal.<sup>62</sup> **Abbreviations:** BSO, buthionine sulfoximine; t-PA, tissue plasminogen activator.

diethyl maleate also induced significant inhibition activity in the aorta.

Another similar study of the blood components in the fibrinolytic system revealed a significant reduction in t-PA activity ( $-29\%$  and  $-22\%$ ) and a significant increase in PAI-1 activity ( $+61\%$  and  $+27\%$ ), following a treatment with BSO or diethyl maleate, respectively. Alpha-2-antiplasmin was not significantly affected by the administration of either GSH-depleting agents.<sup>62</sup>

## Plasminogen/plasmin systems in AD

The plasminogen system is a group of mechanisms whose interaction leads to the production of a protease involved in degrading substrates and avoiding their accumulation, and which is regulated by specific inhibitors.<sup>84</sup> The cerebral plasminogen system does not differ from the systemic plasminogen system, as all the constituents of the systemic mechanism are present in the brain.<sup>85</sup>

Ab is the target for proteolytic degradation by several proteases known as the Ab-degrading proteases.<sup>86</sup> Among the different, best-known Ab-degrading proteases are: neprilysin,<sup>12,87,92</sup> insulin-degrading enzyme,<sup>93,94</sup> endothelin converting enzyme,<sup>93,95,96</sup> matrix metalloprotease,<sup>97–99</sup> and plasmin.<sup>100–102</sup> Of greatest interest to the study of Ab clearance and elimination through the BBB, are neprilysin<sup>79,88,89,103,104</sup> and plasmin.<sup>105</sup>

The plasminogen system is an essential proteolytic mechanism that, by the action of plasmin, effectively degrades Ab peptides (beta-amyloidolysis), prompting us to consider this physiologic process as a preventive mechanism of neurodegenerative processes.<sup>19,106</sup> Nevertheless, the primary substrate of degradation by plasmin in the brain is not very well known.<sup>107</sup> Plasmin activity is diminished in the hippocampus and cortex of patients with AD.<sup>12,108</sup> As mentioned earlier, the excessive production and lack of clearance of the peptide Ab creates the accumulation of senile plaques that define AD.<sup>18</sup>

A decrease in brain plasmin activity leads to a smaller clearance and an increase in the Ab deposits.<sup>15,16</sup> Experimental studies in mice have shown an increase of Ab material deposits and an increase of PAI-1 in the process of AD.<sup>88,89</sup> Therefore, the inhibition of the t-PA by the PAI-1 facilitates the accumulation of Ab material and slows its degradation and later clearance; very important mechanisms in the genesis of AD.<sup>18,102</sup>

For investigators, t-PA plays a significant role in the physiopathology of the central nervous system (CNS).<sup>109</sup> At the CNS level, t-PA has a specific inhibitor, neuroserpin (NSP), which is found in those regions where t-PA is present. The coexpression of NSP and t-PA in the same regions of the brain suggests that NSP is a likely regulator of t-PA activity within the CNS. This complex t-PA/NSP contrasts with the formation of long-lasting, physiologically irreversible complexes between t-PA and PAI-1 (due solely to differences in affinities of t-PA for PAI-1 versus NSP).<sup>110</sup>

In some studies, neurotoxicity has been attributed to the plasminogen system.<sup>111–120</sup> In certain pathologies, such as in ischemia and cytotoxicity, activation of the plasminogen system occurs by t-PA, generating plasmin, which in turn, degrades the extracellular matrix by action on the laminin, producing neuronal loss.<sup>121</sup> Other studies cast doubt on this action of t-PA, demonstrating that direct infusion does not lead to neuronal loss.<sup>114</sup> Conversely, other results have conferred on t-PA a protective characteristic against cellular injury (both *in vitro* and *in vivo*)<sup>122</sup> and considered it to be a regulator of vascular tone and permeability<sup>118</sup> as well as a regulator of the BBB<sup>128</sup> and a mediator in neuronal

connection (synaptic plasticity).<sup>124,125</sup> t-PA has been considered to have effects that are not related to its ability to activate plasminogen. For example, in mice lacking t-PA, neurons were found to be resistant to the damage caused by strokes.<sup>116</sup> Further, t-PA increases microglia activation, without requiring any proteolytic activity.<sup>126</sup> To sum up, t-PA is considered as a cerebral mediator, exercising both proteolytic and nonproteolytic actions, at a metabolic, functional, or morphological level.<sup>127</sup>

The relationship between GSH and the plasminogen system is of great importance for cerebral function. The pharmacological depletion of GSH produces a significant inhibition of the plasminogen mechanism, secondarily inhibiting the generation of plasmin. As indicated previously, the lack of plasminic activity in the brain leads to the accumulation of Ab peptides and to the formation of the extracellular plaques and intracellular tangles, found in AD.<sup>18,106</sup> PAI-1 increases in different pathologies associated with GSH depletion and oxidative stress.<sup>128</sup>

Throughout aging, normal mice have been shown to experience a progressive decrease of t-PA activity.<sup>129,130</sup> In parallel, lower levels of GSH have been found in the cortex, cerebellum, striatum, thalamus, and hippocampus (although hepatic levels remain normal).<sup>35,112,129</sup> This decrease in t-PA activity found in normal mice, is due to a decrease in the expression of t-PA and to an increase in the production of PAI-1, carrying with this an increase in the production of Ab peptides.<sup>114,129,130</sup> The same results have been found in the cerebral tissue of patients with sporadic AD, where a negative correlation between proteolytic activity and the levels of Ab peptides has been observed.<sup>15</sup>

Clinically, there is evidence to suggest that cognitive status can be improved with decreases in the concentration of Ab peptide in the cerebrospinal fluid, and that short- and long-term resistance to cognitive deterioration can be achieved with the administration of heparan sulfate-dermatan sulfate (sulodexide) (a glycosaminoglycan drug that crosses the BBB and acts by inhibiting PAI-1 and activating t-PA, with an accompanying increase in proteolytic activity).<sup>131–143</sup> In a study conducted by us,<sup>144</sup> two groups of patients were treated with sulodexide or with acenocoumarol. Follow up of these patients after 6 years revealed that patients treated with sulodexide experienced significantly less deterioration of cognitive status compared with the group treated with acenocoumarol (Lasierra-Cirujeda, personal communication October, 2011).

In animal models as well as in clinical trials in humans, an enteral or parenteral supply of nutrients, including cysteine, methionine, N-acetyl-cysteine (NAC), and L-2-



oxo-thiazolidine, were found to be suitable precursors for the synthesis of GSH and subsequently led to an increase in the intracellular level of GSH.<sup>28,51,145-147</sup> Normalization of GSH levels improves the proteolytic cerebral capacity. After the administration of GSH to patients with diabetes mellitus type 2, a significant reduction of plasma PA-I levels, and an increment in the concentration of GSH in blood red cells were found. These results suggest the usefulness of GSH in the improvement of the plasminogen system.<sup>148-150</sup>

Finally, the supply of GSH precursors and the resulting increase in the antioxidant GSH has been found to promote cellular resistance to oxidative stress, by leading to an intra- and extracellular proteolytic improvement caused by both the decrease in PAI-1 and the increased clearance of the cerebral Ab proteins.<sup>150,151</sup>

In this review, we hypothesized the importance of the interaction between cerebral GSH and plasminogen systems in neurodegenerative diseases. In summary, the cerebral GSH and plasminogen systems are essential biological processes that combat the neurodegenerative processes that occur more significantly with advancing age. With knowledge of the physiopathology of neurodegenerative processes, both of these systems can be pharmacological targets, providing reason for hope of prevention of neurodegenerative diseases such as AD.

## Disclosure

The authors report no conflicts of interest in this work.

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