

A Study of Glutathione *S*-transferase pi Expression in Central Nervous System of Subjects with Amyotrophic Lateral Sclerosis Using RNA Extraction from Formalin-Fixed, Paraffin-Embedded Material

Ewa Usarek,¹ Beata Gajewska,¹ Beata Kaźmierczak,¹ Magdalena Kuźma,² Dorota Dziewulska,² and Anna Barańczyk-Kuźma^{1,3}

(Accepted June 21, 2005)

The expression of glutathione *S*-transferase pi (GST pi), an enzyme responsible for inactivation of a large variety of toxic compounds was studied in spinal cord, motor and sensory brain cortex obtained from patients who died in the course of amyotrophic lateral sclerosis (ALS). The studies were performed on formalin-fixed, paraffin-embedded (FFPE) and freshly frozen tissues. The method of RNA isolation from FFPE was modified. A significant decrease of GST pi-mRNA expression was found in cervical spinal cord and motor brain cortex of ALS subjects comparing to analogue control tissues ($P < 0.01$), as well as in motor cortex of ALS subjects comparing to their sensory cortex ($P < 0.05$). In spinal cords the decrease in GST pi-mRNA expression was accompanied by a decrease of GST pi protein level. Results indicated lowered GST pi expression on both mRNA and protein levels in the regions of nervous system affected by ALS. The non-properly inactivated by GST toxic electrophiles and organic peroxides may thus contribute to motor neurons damage.

KEY WORDS: Amyotrophic lateral sclerosis; brain cortex; formalin-fixed; Glutathione *S*-transferase pi; paraffin-embedded material; spinal cord.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive degeneration and loss of motor neurons. The disease is familial in 5–10% of cases, the one fifth of which are linked to mutations in SOD gene (1). In about

95% of all cases ALS is a sporadic disorder of unknown origin. Its susceptibility determining factors underlying the neuronal loss remain unclear. The toxicity of environmental factors, excitotoxicity, neurotrophic factors deprivation and oxidative stress, have been suspected to participate in the neuron degeneration (2,3). Glutathione *S*-transferases (GST, EC 2.5.1.18) are a family of enzymes widely distributed in cells. Some of GST isoenzymes express both transferase and selenium-independent peroxidase activity (4,5). They can therefore protect cells against the toxicity of xenobiotics and contribute to antioxidant defense (6). Among the compounds inactivated by glutathione *S*-transferase there are toxic electrophiles such as aliphatic and aromatic heterocyclic

¹ Chair and Department of Biochemistry, Medical University of Warsaw, Banacha 1, 02-097, Warsaw, Poland.

² Department of Neurology, Medical University of Warsaw, Banacha 1, 02-097, Warsaw, Poland.

³ Address reprint requests to: Anna Barańczyk-Kuźma, Chair and Department of Biochemistry, Medical University of Warsaw, 02-097 Warsaw, Banacha 1, Poland. Tel.: +48-22-5720-693; Fax: +48-22-5720-679; E-mail: akuzma@amwaw.edu.pl