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Research Report

# The effect of Zn(II) and streptozotocin administration in the mouse brain

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ABSTRACT

Streptozotocin is a natural antibiotic produced by *Streptomyces achromogenes* able to induce diabetes in experimental animals. Among various toxic properties, streptozotocin is a potent source for reactive oxygen species. In this paper, we report the biological response of brain, upon treatment with streptozotocin in terms of metal ions dismetabolism and metallothionein expression. In addition, important information on the preventive effect of zinc in eliciting the pharmacological effect of the drug are reported, in relation to the effective role of the metal ions in inducing metallothionein synthesis. In the brain, streptozotocin treatment affects mostly the hippocampus and cerebellum as shown by a high GAFP and MT-I-II immunopositivity of glial cells. The Zn pre-treatment reduces significantly, as a general effect, the occurrence of hyperglycaemic status. At the brain level, the observed astrocytosis is strongly reduced. The high inducibility of MT represents a rapid and convenient response able to prevent the deleterious effects consequent to the oxidative stress. All together these results support the efficacy of the Zn treatment in order to prevent streptozotocin effects, including brain tissues.



## 1. Introduction

Streptozotocin (STZ) is a naturally produced antibiotic from *Streptomyces achromogenes*, which is widely used to induce experimental diabetes in mice. Its biological effect lies in the susceptibility of pancreas islets to the drug which enters the cytoplasm via the glucose transporter GLUT2 (Marks et al., 2003). Several deleterious effects of STZ have been reported including DNA methylation (Murata et al., 1999), protein modification (Bidasee et al., 2003) and reactive oxygen species (ROS) generation (Chen et al., 2001; Gille et al., 2002). Pancreatic β-cells are particularly sensitive to oxidative damage by ROS (Quilliot et al., 2005), assigned to the low activity levels of antioxidant enzymes in these cells, whereas the liver and kidney, which also express GLUT2, are more resistant. Structural, morphological and functional brain alterations have been reported in the case of type 1 diabetes. These include activation of cell death pathways after a short focal cerebral ischemia—accelerating the neuronal damage maturation, increasing the infarct volume and inducing post-ischemic

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seizures (Muranyi et al., 2003); macro- and micro-vascular diseases with cerebral atherosclerosis and cognitive decline (Grunnet, 1963; Fergunson et al., 2003).

An important component of the antioxidant protein pool of the cell is represented by metallothionein (MT). This class of proteins, with its low molecular weight and abundance of metal and cysteine, is an efficient radical scavenger due to the presence of cysteine residues that are normally coordinated by Zn(II) and/or Cu(I) ions (Hidalgo et al., 2001; Binz and Kägi, 1997). Four isoforms of these proteins are known in mammals (MT-I-IV) exhibiting, at least in part, tissue specificity: MT-I and MT-II isoforms are expressed in most tissues, MT-III is characteristic of neurons whereas MT-IV is expressed in the keratinising epithelia (Hidalgo et al., 2001).

Zinc is an essential element for the functionality of various biochemical and physiological processes (Tudor et al., 2005) in that it plays a vital role in normal growth, protein metabolism, integrity of membranes, gene expression, wound healing, collagen synthesis, immune system and prevention of apoptosis. Redistribution of total body zinc stores may influence or even control the intensity of the acute phase response (Tudor et al., 2005). Typically MT synthesis can be induced by several metallothioninogenic elements including metal ions like Zn and/or Cu because the promoter region of the multi-gene complex responsible for MT synthesis presents metal-responsive regulatory elements controlled by the metal regulatory transcription factor MTF1 (Radtke et al., 1993; Coyle et al., 2002). MT protects islets from most kinds of ROS (Li et al., 2004a,b). In keeping with this, treating mice with $ZnSO_4$ induces MT also in pancreatic islets, prevents tissue degeneration and protects against STZ-induced diabetes (Ohly et al., 2000). The protective role of MT is also demonstrated by the reduction of STZ-induced DNA damage, degranulation and cell death of pancreatic β-cells in transgenic mice overexpressing MT (HMT-1) upon STZ treatment (Chen et al., 2001), as well as by the extended islet graft survival after hypoxia, again in HMT-1 mice after islet transplantation. An intriguing possibility has arisen recently by Hidalgo's group who have demonstrated that MT is able to directly reduce the inflammatory response associated with CNS injury, leading to an enhanced recovery (Penkowa et al., 2000; Penkowa and Hidalgo, 2001).

In this work, we have addressed the issue of the biological response of brain to STZ because increasing evidence points to an important role of MT in brain in the prevention of oxidative stress and degeneration in relation to the hyperglycaemic status, the two major effects of the drug responsible for diabetes in the animal model. MT are also involved in the brain physiology with a multifunctional role including detoxification against heavy metals, xenobiotics and generation of radical oxygen species, as well as regulation of intracellular Zn(II) and Cu(I) concentrations (Hidalgo et al., 2001; Strausak et al., 2001). Some disorders that are relatively well characterized from the metal physiopathological point of view—Wilson's and Menkes' diseases (Strausak et al., 2001; Hidalgo et al., 2002), as well as the encephalopathies like Alzheimer's, Binswanger's and Pick's diseases and amyotrophic lateral sclerosis involve a strong alteration of brain MT levels (Hidalgo et al., 2002; Zambenedetti et al., 1998, 2002). In particular, significant astroglial MT-I/II immunoreactivity is characteristic of cerebral areas with marked damage of tissues. Production of free radicals in the brain involve catecholamine dismetabolism, specially with a concomitant deficiency of antioxidants, and in the inflammatory phenomena, astrocytes play a central role with MT overexpression (Hidalgo et al., 2002). Thus, an increase in the MT expression level can be considered diagnostic of a tissue-specific response to a functional impairment involving free-radical formation. Oxidative stress is well known to be responsible for brain lesions that characterize neurodegenerative diseases, where the neuroinflammatory processes increase the free-radical production.

Numerous studies have been carried out on the effects of STZ administration on the brain at the biochemical, physiological as well as at the cognitive level. However, the question of possible MT induction in STZ-treated animals and the effects on metal homeostasis has as yet received little attention.

In this paper, we demonstrate that the administration of STZ in mice produces, in the brain as well as in the other target tissues, significant metal ion dismetabolism, as demonstrated by metal and MT quantification in tissues together with an abundant elimination of metals and metallothionein in the urine. In the brain, STZ treatment affects mostly the hippocampus and cerebellum as shown by a high GAFP and MT-I-II immunopositivity of glial cells.

## 2. Results

### 2.1. *Effects of STZ and Zn(II) administration*

The procedure to expose animals to STZ involves a single dose of STZ (220 mg/kg) administered intraperitoneally. The response to the treatment was positive in 80% of experimental animals, among which 40% developed hyperglycaemia (>180 mg/dL plasma) within 48 h post-treatment, 25% between 3–7 days and 15% within 10–30 days. As expected, the affected animals all maintained the hyperglycaemic condition, thus demonstrating the irreversibility of the STZ effect. Thus, all animals of the STZ-treated group were sacrificed 30 days after the ascertained hyperglycaemia. No differences were observed among the control animals sacrificed at different times to ensure age-matching with the STZ-treated specimens.

Fig. 1 shows a typical field of pancreatic islet immunostained for chromogranin A. Panel A, referring to a control animal age-matched with respect to the treated animal, shows the typical morphology of a pancreatic islet. Loss of cells with concomitant diffuse inflammation and infiltration is evident in the STZ-treated animal (Fig. 1B).

In Table 1, some urinary parameters of controls and STZ-treated animals are summarized. Glycosuria increases up to three orders of magnitude with respect to the controls accompanied with a 10- to 30-fold increase in dieresis and an increase in proteinuria.

The supplementation of Zn(II) through drinking water proved to be effective: in the group of animals treated with Zn(II) per os the percentage of cases with hyperglycaemia decreases to 20% up to 30 days after STZ administration. The field shown in Fig. 1C shows that the treatment with Zn(II) does not alter the morphology, yet, as evident in panel 1D, the