The effects of glutathione analogues on Na,K-ATPase activity in the kidneys of the wildtype and the Wfs1 gene mutated mice *in vitro* and *in vivo*

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Protein S-glutathionylation, the reversible formation of mixed disulfides between glutathione and cysteinyl residues of proteins, is a potential mechanism for dynamic, post-translational regulation of a variety of regulatory, structural, and metabolic proteins in cells.[1]

Glutathione (GSH) is the most abundant low-molecular weight thiol compound that occurs in millimolar ranges in the mammalian cells. The compensatory administration of GSH is not effective because of its rapid degradation. Various chemical modifications of the GSH molecule have been carried out to improve its stability and cellular uptake. We have designed and synthesized a small library of tetrapeptidic GSH analogues (UPF peptides). Glutathione analogue UPF1 is a tetrapeptide (o-methyl-L-tyrosine to the N-terminus of GSH). It is shown that this change increased the hydroxyl radical scavenging ability 60-fold compared to GSH itself *in vitro*. UPF17 is a UPF1 with α -glutamate bond instead of γ -glutamate bond. The change of γ -glutamate to the α -glutamate improves the hydroxyl radical scavenging properties.[2]

Na,K-ATPase is a crucial plasma membrane enzyme in kidneys composed of α subunit, β subunit and a regulatory subunit belonging to the FXYD protein family, which all contain free cysteine residues. An α subunit has 23 free cysteine residues, the β has only one and FXYD protein two of them. The glutathionylation of the Na,K-ATPase causes a decrease in the Na,K-ATPase activity.[3,4]

The WFS1 gene encodes wolframin, an endoplasmic reticulum membrane glycoprotein, which regulates protein folding and secretion, cellular transport and calcium homeostasis. Mutations in WFS1 gene lead to changes in Na,K-ATPase activity and in the expression of the α and β subunits.[5]

The aim of this study was to investigate the effect of GSH and its analogues UPF1 and UPF17 on Na,K-ATPase activity in two months old wildtype and WFS1 knockout mice kidney *in vitro* and *in vivo*.

The activity of the Na,K-ATPase was determined by Innova Biosciences kit as the ability to hydrolyze ATP and expressed as $\mu\text{mol Pi/mg min}$. *In vitro* the samples were pre-incubated with GSH and UPF peptides for 10 and 45 minutes at room temperature. *In vivo* glutathione analogues were administrated i.p. 1 mg/kg for 5 days.

The activity of Na,K-ATPase in two months old wildtype and WFS1 knockout mice kidney was measured (Figure 1). The study showed that glutathione and UPF17 in concentrations 10^{-3} M inhibit the activity of Na,K-ATPase in wildtype and WFS1 knockout mice kidney in a time dependent manner (Figure 2). UPF1 in concentrations 10^{-3} M at pre-incubation time 10 min inhibits the activity of Na,K-ATPase in wildtype, but activates in WFS1 knockout mice kidney (Figure 3). Same concentration of UPF1 at pre-incubation time 45 min did not have any effect in both genotypes *in vitro*. Intraperitoneal administration of UPF1 and UPF17 inhibit the activity of Na,K-ATPase in wildtype mice kidney, but did not have any effect in WFS1 knockout mice *in vivo* (Figure 4).

Figure 1: The activity of Na,K-ATPase in two months old wildtype and WFS1 knockout mice kidney. * $p < 0.05$; $n = 8$.

Figure 2: The modulation of Na,K-ATPase activity by GSH and UPF17 (1 mM) in two months old wildtype mice kidney in vitro. Pre-incubation time 10 and 45 minutes. The basal Na,K-ATPase activity is 1.0. Values are represented as mean $\pm$ SEM; $n = 8$.

Figure 3: The modulation of Na,K-ATPase activity by GSH, UPF17 and UPF1 (1 mM) in two months old wildtype and WFS1 knockout mice kidney in vitro. Pre-incubation time 10 (A) and 45 (B) minutes. The basal Na,K-ATPase activity is 1.0. Values are represented as mean $\pm$ SEM. * $p < 0.05$; $n = 8$.

Figure 4: The activity of Na,K-ATPase in two months old wildtype and WFS1 knockout mice kidney after intraperitoneal administration of UPF1 and UPF17 (1 mg/kg, 5 days). Values are represented as mean $\pm$ SEM. * $p < 0.05$; The basal Na,K-ATPase activity is 1.0; $n = 8$.

The various effects of glutathionylation on the activity of Na,K-ATPase may be caused by structural differences between the GSH and UPF peptides (the addition of a methylated tyrosine moiety to the GSH backbone and the change of the peptide bond type). Substitution of the γ -glutamyl with α -glutamyl moiety drastically improved the effect on Na,K-ATPase activity of UPF peptides. As well as by conformational changes in the enzyme of WFS1 knockout mice (different access and affinities of thiol groups of the enzyme).

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