Original Article

In Vivo Imaging of Renal Redox Status during Azelnidipine Treatment

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The effect of the calcium channel blocker azelnidipine on the redox status of a murine hypertension model was analyzed and imaged using *in vivo* low frequency electron paramagnetic resonance (EPR). A murine two kidney–one clip (2K1C) hypertension model was produced by a clipping of the right renal artery. The resulting hypertensive mice were treated with low-dose azelnidipine (1 mg/kg/d), with high-dose azelnidipine (3 mg/kg/d) or without azelnidipine (HT group). An EPR system equipped with a loop-gap resonator and an imaging system was employed. Redox status was evaluated as organ reducing activity measured by means of the decay rate (half-lives) of the spin probe 3-carbamoyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (Carbamoyl-PROXYL). Four weeks after clipping the mice demonstrated hypertension as expected. After the additional 2 weeks of azelnidipine treatments, the Carbamoyl-PROXYL half-lives of the Low and High azelnidipine groups measured in the upper abdominal area were significantly shorter than those of the HT group, suggesting improvements in the reducing activity. The blood pressures of the three groups showed no significant differences at this time, and there was no correlation between the renal reducing activity and either blood pressure or serum creatinine values. EPR imaging studies revealed that the improvement in abdominal reducing activity was mainly recognized in the kidney but not in the liver. These results indicate that azelnidipine ameliorates renal redox status through an improvement in reducing activity independent of blood pressure control. (*Hypertens Res* 2008; 31: 1643–1650)

***Key Words*: oxidative stress, imaging, azelnidipine, electron paramagnetic resonance**

Introduction

A constant stream of studies alleged an important role of reactive oxygen species (ROS), especially superoxide anion ($O_2^{\cdot -}$), in the pathogenesis of hypertension. As this allegation become more widely recognized, the importance of analyzing the kinetics of ROS or related substances *in vivo* has increased. However, it is still difficult to measure the concurrent *in vivo* redox status non-invasively because ROS related reactions generally have high rate constants and they are often estimated indirectly by measuring the end-products of lipid, protein or gene oxidation. Inevitably, problems concerning the resemblance to and reproduction of *in vivo* circumstances are continuously open to criticism in these *in vitro* or *ex vivo* estimations (*1*). In the field of hypertension research, these problems make it difficult to distinguish the antioxidative effects of the drugs from their effects on blood pressure or hemodynamics (*2*, *3*).

Electron paramagnetic resonance (EPR) has the potential for *in vivo* direct measurement of redox status. Applications of EPR for *in vivo* measurements has been difficult because 1)

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