# Protective Effects of Hydrogen-rich Medium on Schwann Cells Apoptosis Induced by High Glucose

**Authors:** Yang Yu ,Yang Jiao, Bo Li, Yonghao Yu

Department of Anesthesiology, General Hospital of Tianjin Medical University, Tianjin. 300052

**Introduction:** Diabetic peripheral neuropathy (DPN), affecting up to 50% of patients with diabetes, is one of the most prevalent and debilitating long-term microvascular complications of diabetes, and no effective therapy exists. DPN affects both sensorimotor and autonomic parts of the peripheral neural systerm (PNS), and the most representative clinically recognized form is diabetic sensorimotor polyneuropathy (DSPN) which typical symptoms are pain, paraesthesia and sensory loss. Previous studies have demonstrated that oxidative stress and Poly (ADP-ribose) polymerase-1 (PARP-1) whose activation in neurons and Schwann cells of the peripheral nerve may be the unifying factor for the damaging effect of hyperglycemia. The aim of this study was to investigate the protective effects of hydrogen-rich medium on the high glucose-induced oxidative stress, PARP-1 pathway activation and Schwann cells (SCs) apoptosis in vitro.

**Methods:** Primary Rat Schwann cells were purchased from Sciencell Corporation. The cultured SCs were treated in duplicate consistently with 5.6 mmol/L of glucose as the control (Con), with 50 mmol/L of glucose as high glucose (HG) group, with 0.6 mmol/L hydrogen-rich medium as hydrogen ($H_2$) group, with HG in the presence of 0.6 mmol/L of hydrogen-rich medium for 48h, respectively. And treating the cells with 44.4 mmol/L mannitol plus 5.6 mmol/L glucose as high osmotic control. Cell viability and apoptosis were evaluated through CCK-8 and Annexin V/PI assay, respectively; Concentration of 8-hydroxy-2-deoxy Guanosine (8-OHdG) and $ONOO^-$ was detected by Elisa; Intracellular oxygen free radicals (ROS) was confirmed by flow cytometry analysis. Colorimetric assays was performed to analyze the activity of Caspase-3 and western blot was performed to analyzed the expression levels of PARP-1,cleaved PARP-1, PAR, AIF,Bax,and Bcl-2. All data were expressed as the means±SEM and the difference among groups was analyzed by one-way ANOVA. All tests were performed using the statistical analysis software SPSS 21.0.

**Results:** We found that high glucose could induce severe oxidative stress and promoted both caspase-dependent and caspase-independent apoptosis of SCs, treatment with hydrogen-rich medium inhibited the HG-induced oxidative stress by reducing ROS and $ONOO^-$ production, 8-OHdG levels, Caspase-3 activity and apoptosis in SCs. Furthermore, treatment with hydrogen-rich medium down-regulated the HG-induced release of PAR, cleaved PARP-1 expression and AIF nuclear translocation, but up-regulated the Bcl-2 expression in SCs.

**Conclusions:** Our results indicated that hydrogen-rich medium inhibited the HG-induced oxidative stress-induced apoptosis of SCs in both caspase-dependent and caspase-independent pathways, seems to be an effective buttress of treatment for DPN that can largely improve the quality of patients' life.