Effect of superoxide anion scavenger on cardiac apoptosis in rat with chronic intermittent hypoxia

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Background. Very limited information regarding the protective effects of superoxide anion scavenger on intermittent hypoxia-induced cardiac apoptosis in sleep apnea was available. The purpose of this study was to evaluate the effects of superoxide anion scavenger on cardiac Fas-dependent and mitochondria-dependent apoptotic pathways in rat with sleep apnea model.

Methods. Twenty-four SD rats were divided into three groups, rats with normoxic exposure (Control) and rats with nocturnal intermittent hypoxia (HYPOXIA, 3-7%O_2 versus 20%O_2 per 40 seconds cycle, 8 hrs per day, 1 month), and rats with pre-treated superoxide anion scavenger (5 mg/kg, i.p. per day) under nocturnal intermittent hypoxia (HYPOXIA- O^2- SCAVENGER) at 5-6 months of age (HYPOXIA- O^2- SCAVENGER). After administration of superoxide anion scavenger, the excised hearts were measured by positive TUNEL assays and Western Blotting.

Results. Superoxide anion scavenger decreased HYPOXIA-induced cardiac TUNEL-positive apoptotic cells. Superoxide anion scavenger decreased HYPOXIA-induced Fas ligand, Fas death receptors, Fas-associated death domain (FADD), activated caspase 8, and activated caspase 3 (Fas pathways). Superoxide anion scavenger decreased HYPOXIA-induced Bad, Bax, Bax-to-Bcl2 ratio, activated caspase 9, and activated caspase 3 (mitochondria pathway).

Conclusions. Superoxide anion scavenger prevented long-term intermittent hypoxia-enhanced cardiac Fas-dependent and mitochondria-dependent apoptotic pathways in rat models. Our findings imply that suppression of superoxide free radicals will prevent delirious cardiac apoptosis in chronic intermittent hypoxia.

Experimental Procedures

Isolated Rat Hearts

Animals were killed by cervical dislocation, and heart was removed, weighed, washed in ice-cold PBS buffer and the left ventricle was removed. Left ventricle tissue were freeze at the indicated times for the preparation of extracts for immunoblotting.

TUNEL assay

The 0.2-μm thick paraffin sections were cut from paraffin-embedded tissue blocks. The tissues sections were put into Hybridization Incubator 58℃ overnight, and deparaffinized by immersing in 100% xylene. Then rehydrated through graded alcohols and soaked in ddH_2O. The sections were incubated with proteinase K, washed in phosphate-buffered saline, incubated with permeabilisation solution, blocking buffer, and then washed two times with PBS. The terminal deoxynucleotidyl transferase and fluorescein isothiocyanate-dUTP for 60 min at 37 ℃ using an apoptosis detection kit (Roche Applied Science, Indianapolis, IN, USA) for detection. TUNEL-positive nuclei (fragmented DNA) fluoresced bright green at 450-500 nm. The mean number of TUNEL-positive cells were counted for at least 5-6 separate fields x 2 slices x 3 regions of the left ventricle (upper, middle, lower) excised from six rat hearts in each group. All counts were performed by at least two independent individuals in a blinded manner.

Western blotting

Samples of frozen brain were homogenized in ice-cold lysis buffer, using an Polytron homogenizer and then were harvested by centrifugation. Protein homogenates were subjected to analysis in 12% SDS-PAGE. After the electrophoresis, the proteins were blotted onto a PVDF membrane in blotting chamber at a current of 150 mA for 2 hours. The blotted membranes were blocked with 5% non-fat milk in TBS buffer at room temperature for 1hour and then probed with diluted antibodies at 4℃ for overnight. The blots then followed by 3 times of washes with the TBS buffer and reported the membranes with appropriate horseradish peroxides conjugated secondary antibodies in TBS buffer at room temperature for 2 hours. The membranes were finally washed 3 times as previous described and signals were developed using western blotting Lumino Reagent according the manufacturer's instructions.

To investigate the hypoxia induced cardiac apoptosis, in TUNEL assay.

Fig.1

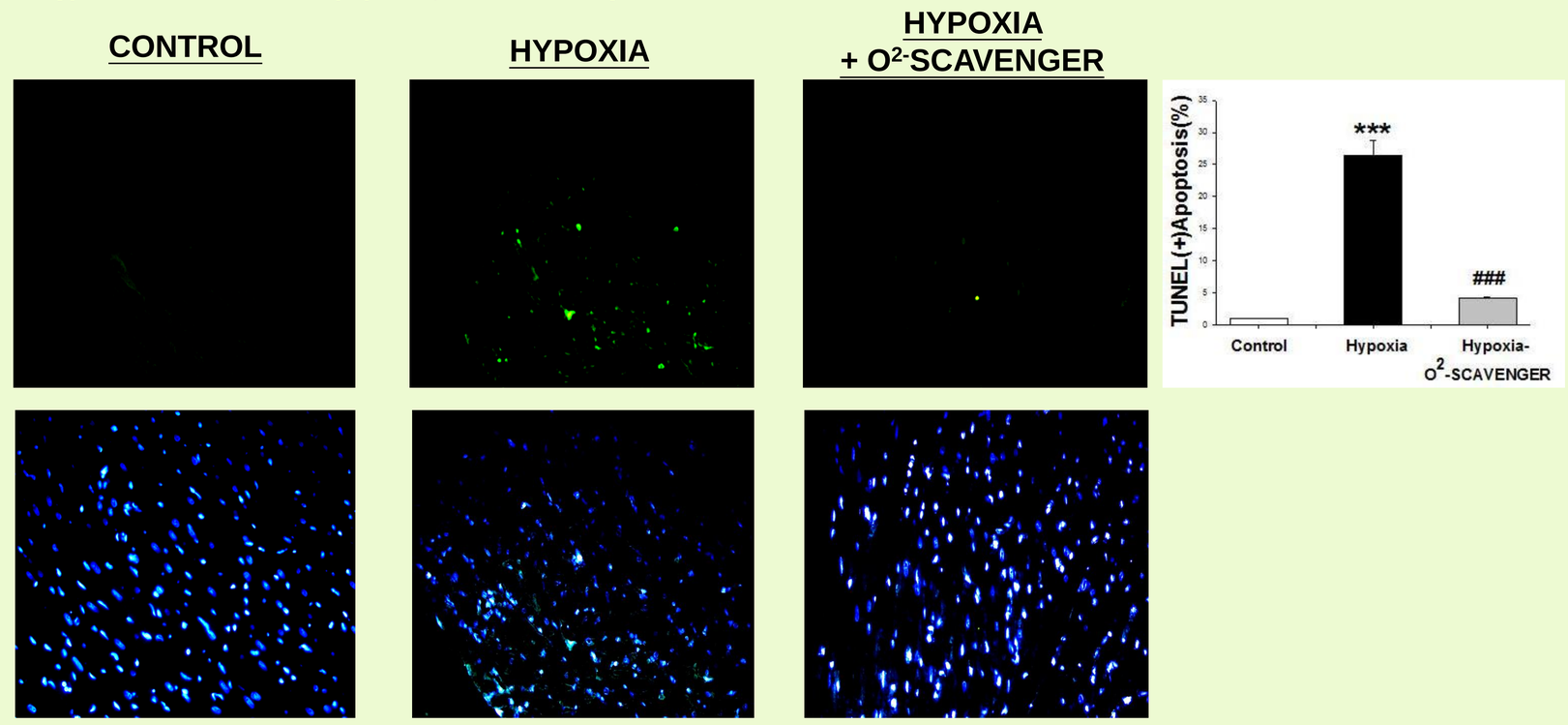
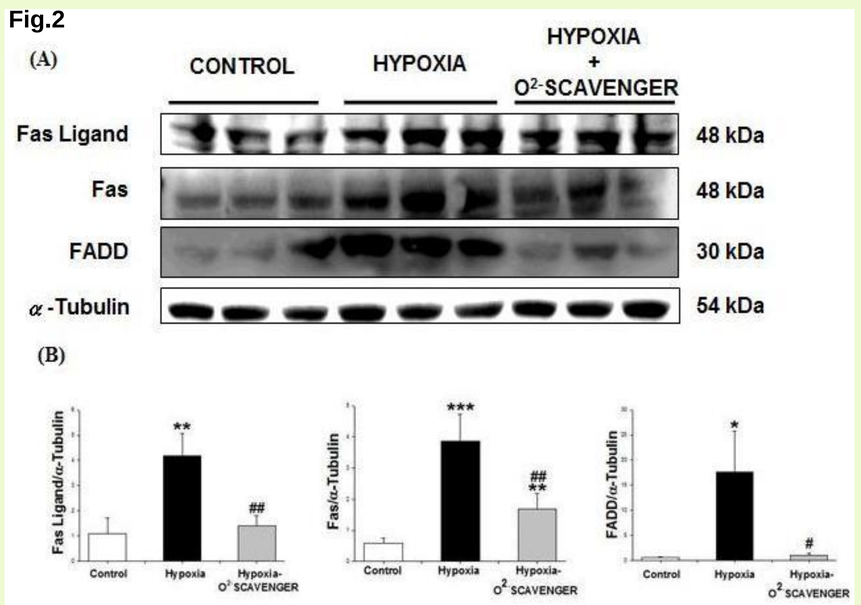


Fig. 1 Hypoxia triggered cardiac apoptosis and exercise attenuated the TUNEL positive cell. The red arrows points out the apoptotic cell.

In order to investigate the hypoxia induced cardiac apoptosis, and it is mediated by which apoptosis pathway?

Fig.2



Whether the hypoxia induced cardiac apoptosis influence by survival pathway?

Fig.4

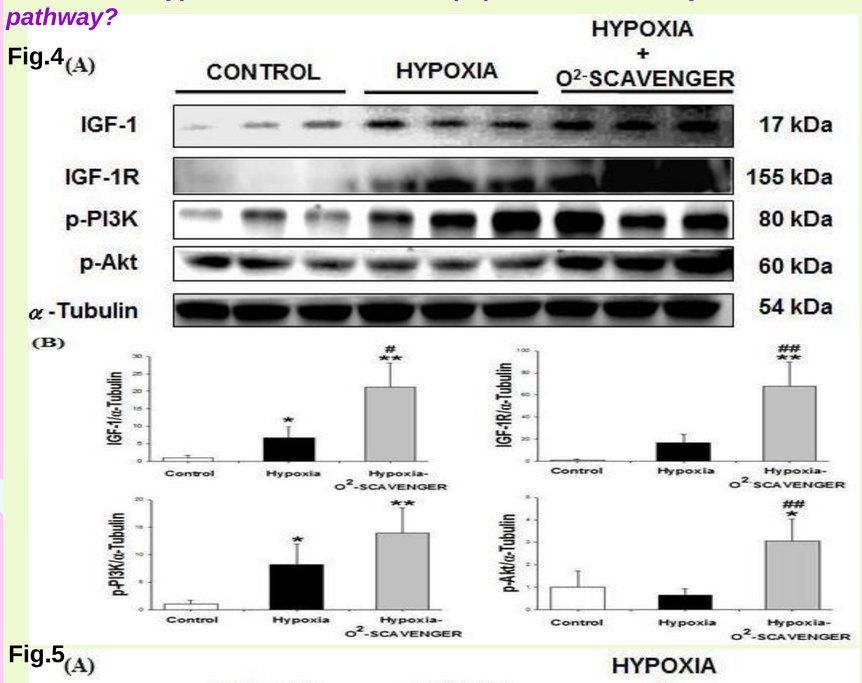


Fig.5

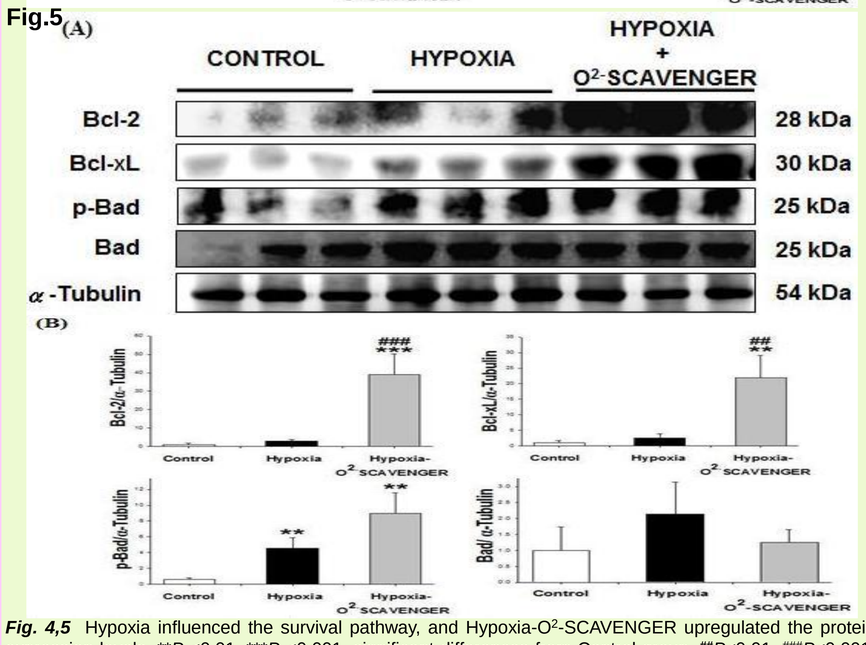


Fig. 4,5 Hypoxia influenced the survival pathway, and Hypoxia-O^2-SCAVENGER upregulated the protein expression levels. $^{**}P$ <0.01, $^{***}P$ <0.001, significant differences from Control group. $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001, significant differences between Hypoxia group and Hypoxia-O^2-SCAVENGER group.

Fig.3

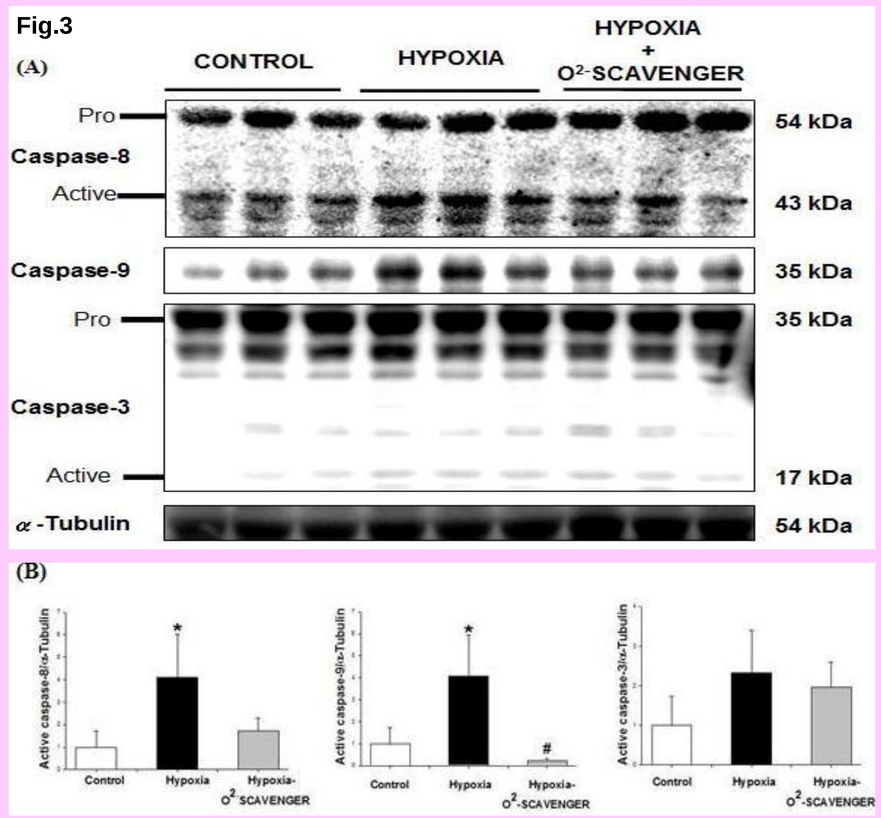


Fig. 2,3 Hypoxia induced cardiac apoptosis was mediated by Fas-dependent apoptosis. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 significant differences from CONTROL group. $^{\#}P$<0.05, $^{\#\#}P$<0.01, significant differences between HYPOXIA group and HYPOXIA-O^2-SCAVENGER group

Summary

Fig.6