Supplemental figure legend

Superoxide anion radical (O$_2^\cdot$) scavenging activity in mitochondria

An ESR spectrometer (JES-FR 30; JEOL, Tokyo, Japan) equipped with manganese oxide (MnO) as an internal standard was used for measuring O$_2^\cdot$ scavenging activities. The conditions for ESR were as follows: magnetic field: 335±5 mT; power: 4 mW; modulation width: 0.079 mT; modulation amplitude: 1×0.1 mT; response time: 0.1 sec; amplitude: 1×200; sweep width: 5.000 mT; and sweep time: 2 min. The method for spin trapping of the O$_2^\cdot$ radical was based on previous studies.\(^1\) To prepare the solution, a mixture was added containing 50 µl of 5 mM hypoxanthine (Sigma Chemical), 15 µl of 5,5'-dimethyl-1-pyrroline-N-oxide (DMPO, Labotec Co. Tokyo, Japan), 50 µl of sample (n=10, each) or superoxide dismutase (SOD)-bovine erythrocytes (Calbiochem, Inc., La Jolla, CA), 50 µl of 0.4 units/ml xanthine oxidase (XOD) (Roche K. K., Tokyo, Japan). After mixing well, the reaction mixture was transferred onto a quartz flat cell, and monitoring of the ESR spectrum was started exactly 1 min after the addition of XOD. All solutions were prepared with 0.1 M potassium phosphate buffer (pH 7.4). %SOD activity in mitochondria was shown as [(DMPO-O$_2^\cdot$ signal before administration of sample - DMPO-O$_2^\cdot$ signal after administration of sample)/DMPO-O$_2^\cdot$ signal before administration of sample] x 100 (%). Values were expressed as the means±S.E.M. n=12 each, *p <0.01 vs. young mice, *p <0.01 vs. old mice.

Typical ESR spectra of DMPO-O$_2^\cdot$ at various ages of mitochondria. The attenuation of the signal by SOD shows the component dependent on O$_2^\cdot$ (Left panel). SOD activity was decreased in mitochondria from old mice compared to that from young mice. Treatment of MitoTEMPO improved the SOD activity in old mice (Right panel).