A Self-assembled Antioxidants Nanoparticle Enhances Exercise Performance in High-intensity Running

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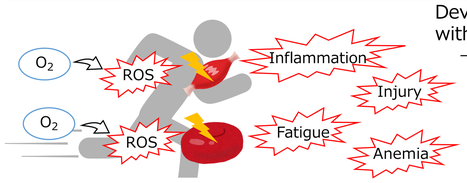
Introduction

Exercise performance and Reactive oxygen species (ROS)



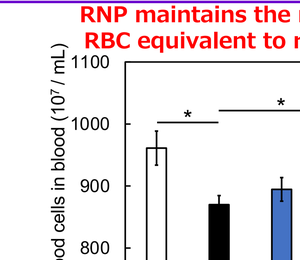
Deviation of about 10 years between average life expectancy and healthy life expectancy with no need for nursing care , *etc.*.
→Loss of exercise performance is one of the main cause.

Why does exercise performance decline?
→One of the factor is **Reactive Oxygen Species (ROS)** (*e.g.* O_2^-, HO·)

ROS is produced by respiration and increases explosively during exercise.
ROS denatures muscles and red blood cells, causing inflammation and damage.
ROS interferes with biological functions and reduces exercise performance.

Exercise-induced ROS damages the body and inhibits exercise performance.
Antioxidant capacity declines with age, therefore it is important to improve external antioxidant capacity.

What are antioxidants and what are problems of antioxidants?



Antioxidant drugs that remove ROS are expected to improve exercise performance.
However, the effect was not as great as expected.

Common antioxidants are low molecular weight (LMW) (*e.g.* ascorbic acid, coenzyme Q10).
LMW antioxidants diffuse through the body in a non-specific and instantaneous manner.

✓Quickly eliminated
Cannot scavenge ROS generated by exercise
✓Mitochondrial dysfunction
ROS is used in the electron transfer system involved in energy production
Inhibits energy production by removing ROS used in mitochondria.

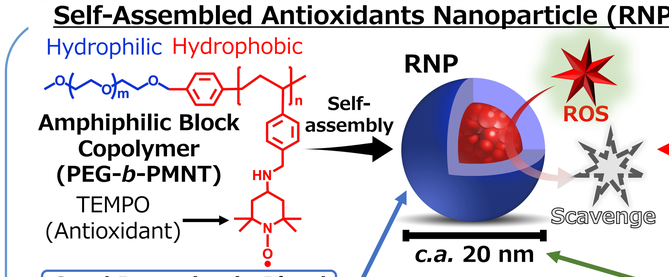
Side effects ≧ Effects (Overdose syndrome)

LMW antioxidants worsen rather than improve exercise performance.

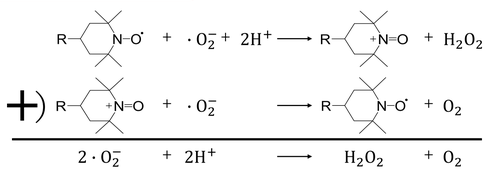
Strategy

It is needed new antioxidant that selective affect and long-retention without side effects.

Self-Assembled Antioxidants Nanoparticle (RNP)

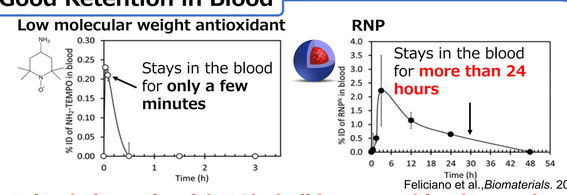


Antioxidant Effect



Nitroxide radicals catalytically scavenge ROS

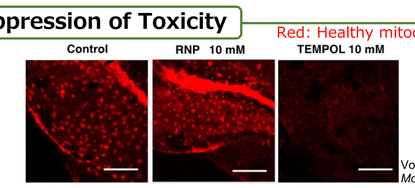
Good Retention in Blood



Feliciano et al.,*Biomaterials.* 2017

Polyethylene glycol (PEG) shell improves blood retention

Suppression of Toxicity



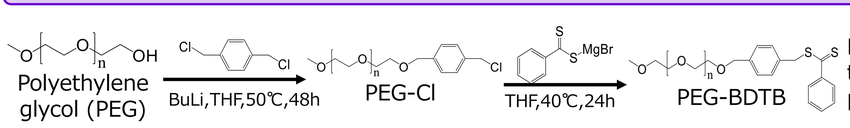
Vong et al., *Mol Pharm.* 2016

Nano size inhibits uptake by normal cells
Suppression of mitochondrial dysfunction

Could RNP with these characteristics improve exercise performance?

Preparation of Self-assembled Antioxidants Nanoparticle (RNP)

Synthesis of Amphiphilic Block Copolymer



BuLi: n-Butyllithium, THF: Tetrahydrofuran, AIBN: Azobisisobutyronitrile, DMF: *N*,*N*-dimethylformamide

Scheme 1: Synthesis of amphiphilic block copolymer (PEG-*b*-PMNT)

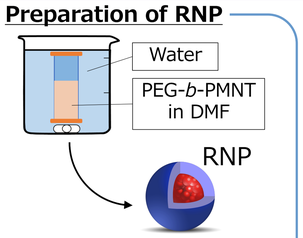
Nuclear Magnetic Resonance (NMR) to determine the structure of each polymer

Gel Permeation Chromatography (GPC) to determine the molecular weight of each polymer

Electron Spin Resonance (ESR) to confirm the introduction of nitroxide radicals in PEG-*b*-PMNT

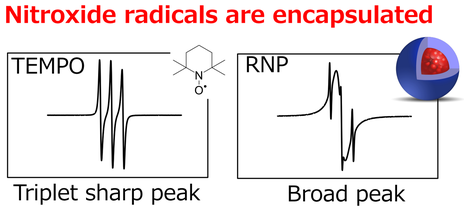
Making Self-assembled Antioxidants Nanoparticle (RNP)

Preparation of RNP



PEG-*b*-PMNT in DMF dialysis against to water

Nitroxide radicals are encapsulated



TEMPO: 2,2,6,6-tetramethylpiperidine 1-oxyl

Fig.1: The ESR spectra of TEMPO (left) and RNP (right)

Nano-sized particles with single-peak

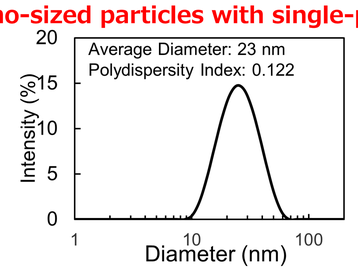


Fig.2: The size distribution of the RNP analyzed by the dynamic light scattering (DLS) measurement

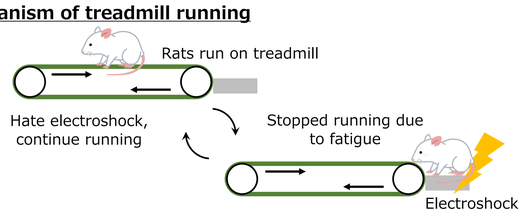
Evaluation of the Effect of Self-Assembled Antioxidants Nanoparticle (RNP) on Improving Exercise Performance

RNP Improves Exercise Performance Whether Administered Subcutaneously or Orally

Treadmill Running

Fischer344, 10 weeks old, male
Rats acclimated to treadmill running were administered the substances listed on the right subcutaneously or orally.

Mechanism of treadmill running

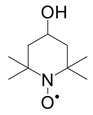


Each different individual was used to run the treadmill with high-intensity (40m/min).
Assessment of exercise performance: To exhaustion
Assessment of RNP effect mechanisms: 10, 30, 50 min

Group	Rest or Run	Administered Substances
Rest	Not run(rest)	PBS**
Vehicle (Control)	Run	PBS
TEMPOL (LMW* antioxidant)	Run	TEMPOL***
RNP	Run	RNP

*LMW: Low Molecular Weight
**PBS: Phosphate-buffered saline
***TEMPOL: 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl

TEMPOL

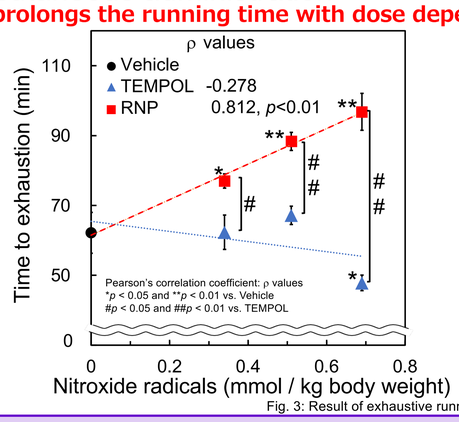


TEMPOL and RNP were administered at the same concentration based on nitroxide radicals, which have antioxidant capacity.

Subcutaneous administration
Running to exhaustion: 0.34 - 0.69 mmol/kg body weight
10, 30, 50 min run: 0.51 mmol/kg body weight
Oral administration (free-drink 1day)
Running to exhaustion: *c.a.* 2.5 mmol/kg body weight

A: Subcutaneous administration

RNP prolongs the running time with dose dependent



B: Oral administration (Free-drink)

RNP prolongs the running time even by free-drink

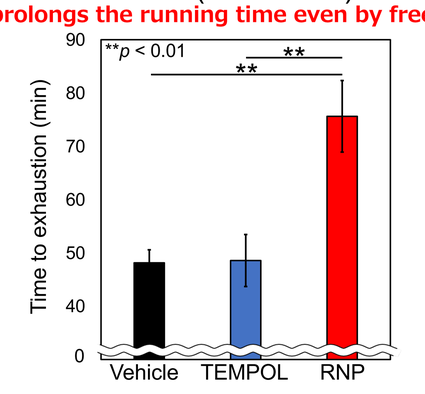


Fig. 3: Result of exhaustive running after (A) subcutaneous and (B) oral administration

Protective Effect on Red Blood Cell (RBC)

RNP maintains the number of RBC equivalent to rest group

Fig. 4: The number of countable RBC after 50 min running.

RNP inhibits exercise induced-hemolysis

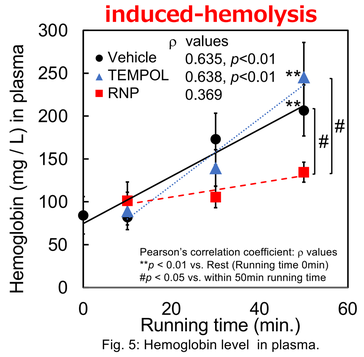


Fig. 5: Hemoglobin level in plasma.

RNP suppresses the release of iron, which causes oxidative stress.

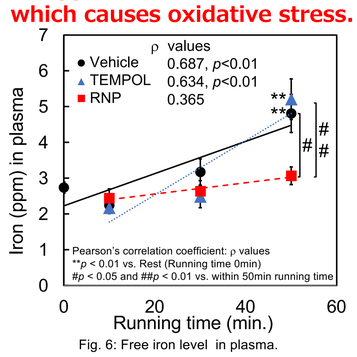


Fig. 6: Free iron level in plasma.

RNP inhibits oxidative stress in RBC

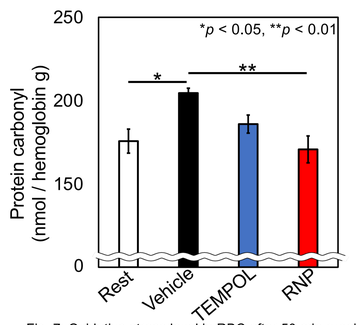


Fig. 7: Oxidative stress level in RBC after 50 min running.

RNP protects RBC from being weakened by running

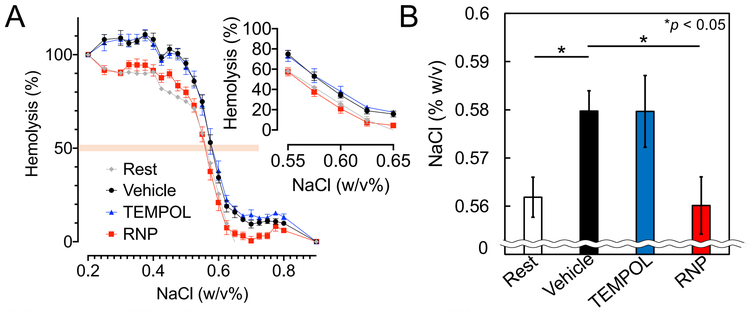


Fig. 8: Osmotic fragility of RBC after 50 min of running. (A) Hemolyzed fraction of the RBC in response to the NaCl concentration. Small graph focuses on the (A) between 0.55% and 0.65% of NaCl (w/v%). (B) Half-hemolytic concentrations of the NaCl, shown as orange in (A).

Protective Effect on Skeletal Muscle

RNP inhibits oxidative stress in skeletal muscle

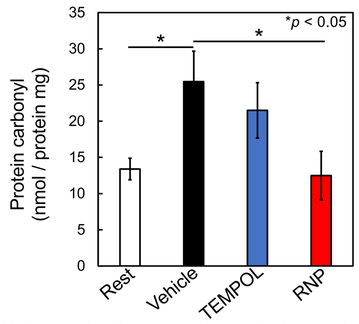


Fig. 9: Oxidative stress level in gastrocnemius muscle after the exhaustive running.

RNP reduces exercise induced-muscle damage

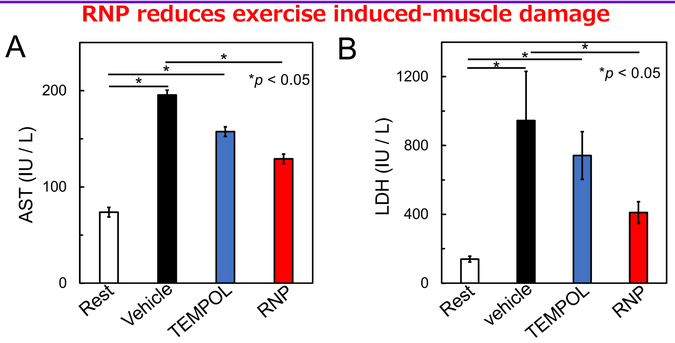
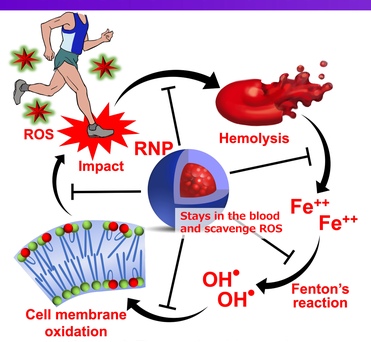


Fig. 10: Muscle damage marker in plasma; (A) aspartate aminotransferase (AST), (B) lactate dehydrogenase (LDH)

Discussion and Conclusion



Scheme2: The negative vicious cycle is caused by high-intensity running

High-intensity running causes a negative vicious cycle, as shown in Scheme 2

1. High-intensity running induces reactive oxygen species (ROS).
2. Red blood cell (RBC) become fragile due to oxidative stress, and the stimulus of exercise (impact of the foot, contraction of muscles and blood vessels) caused them to be break (hemolysis), releasing iron into the bloodstream.
3. The released free-iron increases oxidative stress *via* the Fenton reaction.
$$(Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-)$$
4. Continuous high-intensity running further induces ROS and causes oxidative stress to RBC and then enhances hemolysis and releasing free-iron in the blood.

The steps 1 to 4 further destroys the RBCs and reducing the oxygen-carrying capacity
→**The rats become exhausted and cannot run.**

The self-assembled antioxidants nanoparticle (RNP), which possesses a long-term blood circulation tendency, continuously eliminated the ROS in the blood to break the vicious cycle shown in Scheme 2.
→**RNP prolongs the running time of the rats with dose-dependent without side effects of overdose caused by low molecular weight antioxidant (TEMPOL).**

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