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Press Release

Keio University Faculty of Pharmacy
Nara Medical University
Sojo University DDS Research Institute

Antidote Against Cyanide Poisoning Developed from Artificial Red Blood Cells

–Efficacy in the treatment of cyanide poisoning with both fast-acting and versatile antidote–

A research group at the Keio University Faculty of Pharmacy, Nara Medical University, and the Sojo University DDS Research Institute has developed liposome-encapsulated (Note 1) methemoglobin (Note 2) as an antidote against cyanide poisoning. These findings come from a research group led by Yuto Suzuki, a first-year doctorate student at the Keio University Graduate School of Pharmaceutical Sciences; Associate Professor Kazuaki Taguchi and Professor Kazuaki Matsumoto at the Keio University Faculty of Pharmacy; Professor Hiromi Sakai at Nara Medical University; and Specially Appointed Professor Masaki Otagiri at the Sojo University DDS Research Institute.

Cyanide can be found in everyday products such as metal-plated goods, insecticides, pesticides, and insulation (synthetic resin). At the same time, cyanide is highly poisonous and induces lethal poisoning when inhaled or ingested in large quantities. Cyanide poisoning occurs around the world under various circumstances, such as inhalation of cyanide-containing smoke created from burning buildings, accidental ingestion, and suicide. Nitrites (Note 3) are currently approved as cyanide poisoning antidotes. However, nitrites, which express a detoxification effect by converting hemoglobin in red blood cells to methemoglobin (Note 4), have been unable to achieve rapid detoxification due to how long methemoglobin conversion takes. Furthermore, nitrites cannot be used to treat cyanide poisoning resulting from a fire (accompanied by carbon monoxide poisoning) due to the reduced oxygen-carrying capacity of red blood cells that have undergone methemoglobin conversion. For these reasons, it is paramount to improve the speed and versatility of cyanide poisoning treatment via nitrites.

In this project, researchers developed a novel cyanide poisoning antidote consisting of liposome-encapsulated methemoglobin (metHb@Lipo), which was obtained by oxidizing the hemoglobin contained within an artificial red blood cell preparation (Note 5). metHb@Lipo was designed with concept of artificially reproducing nitrites' antidotal mechanism that captures and detoxifies cyanide in the body by oxidizing hemoglobin into methemoglobin in red blood cells. In practice, metHb@Lipo bound with high affinity to cyanide, and increased survival among mouse models for lethal cyanide poisoning. Beyond extending the life of mouse models for lethal cyanide poisoning, metHb@Lipo was also more effective than nitrites in rapid detoxification and improving tissue hypoxia.

These outcomes demonstrated that metHb@Lipo is a more potent, rapid, and versatile antidote to cyanide poisoning than nitrites. The research team hypothesizes that this rapid

detoxification was made possible because metHb@Lipo captures cyanide without needing to spend time converting hemoglobin into methemoglobin. They also believe that because metHb@Lipo does not convert hemoglobin to methemoglobin, the oxygen-carrying capacity of red blood cells is maintained, preventing hypoxia. Thus, metHb@Lipo can be used even when cyanide poisoning occurs in situations where nitrites are contraindicated (i.e. smoke inhalation from a fire), and its clinical use as a novel antidote to cyanide poisoning is expected to expand treatment options moving forward.

The results of this research were published in the September edition of the international journal on science and technology *Journal of Controlled Release* (digital edition published on July 15).

1. Main Points of Research

- metHb@Lipo was encapsulated methemoglobin in a lipid membrane.
- metHb@Lipo artificially reproduced the detoxification mechanism of existing nitrite preparations.
- metHb@Lipo dramatically increased survival, and proved more effective than existing nitrite preparations in mouse models for lethal cyanide poisoning
- metHb@Lipo showed greater immediate detoxification efficacy than nitrites.
- Administration of metHb@Lipo showed a detoxifying effect on cyanide poisoning without inducing hypoxia.

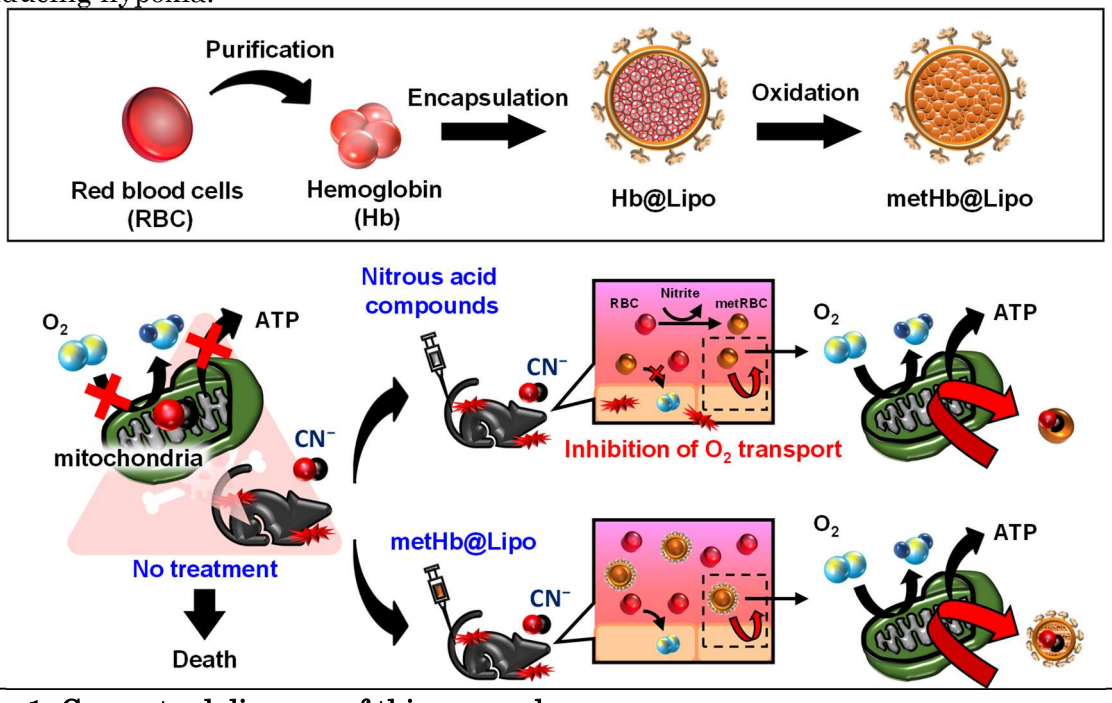


Figure 1. Conceptual diagram of this research

Cyanide binds to cytochrome c oxidase in intracellular mitochondria and inhibits mitochondrial respiratory function. Nitrite-oxidized red blood cells (methemoglobin) have the effect of extracting cyanide from mitochondria and restoring their respiratory function, come with noted complications such as a decrease in oxygen-carrying capacity.

Nitrites are added to artificial red blood cells (hemoglobin vesicles) in which hemoglobin

obtained from human red blood cells is encapsulated in a lipid membrane. The encapsulated hemoglobin is then oxidized to methemoglobin, which has a high binding affinity to cyanide, to produce metHb@Lipo. Like nitrites, metHb@Lipo improves the symptoms of poisoning by restoring mitochondrial function, which is central to the cyanide poisoning mechanism. However, unlike nitrites, it prevents the onset of hypoxia.

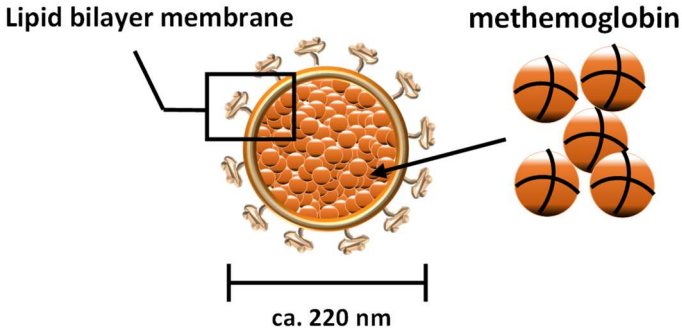
2. Research Background

Cyanide is essential to chemical industries, but it is also a deadly poison that can be lethal when inhaled or ingested in large amounts. Every year a large number of cyanide poisoning cases are reported around the world. Cyanide poisoning occurs under various circumstances including suicide, accidental ingestion, attempted homicide, and inhalation of cyanide gas created when heating insulation derived from synthetic resin (a new construction material) burns. Cyanide poisoning requires swift treatment via an antidote, but the nitrites currently used have not proven to have a fast-acting effect. This is due to the time required to produce the detoxification agent methemoglobin. Nitrites exert a detoxifying effect by oxidizing the hemoglobin in red blood cells present in the blood to methemoglobin, which, in turn, binds to cyanide. Furthermore, the use of nitrites is contraindicated when cyanide poisoning occurs at the site of a fire with possible concurrent carbon monoxide poisoning. This is because the process of converting hemoglobin in red blood cells into methemoglobin leaves hemoglobin unable to execute its original function of carrying oxygen.

This research was begun to develop a cyanide poisoning antidote that would address the challenges facing the clinical use of nitrites. In order to achieve this, researchers posited that cyanide detoxification of nitrites could be replicated without converting hemoglobin in red blood cells to methemoglobin through the administration of liposome-encapsulated methemoglobin (metHb@Lipo), which has already been oxidized to inner hemoglobin of hemoglobin vesicles (a cellular type of artificial red blood cells).

3. Content of Research and Results

First, hemoglobin vesicles (artificial blood created from purified human red blood cells encapsulated in a lipid bilayer) were mixed with sodium nitrite, oxidizing the contained hemoglobin to produce metHb@Lipo. The resulting metHb@Lipo comprised of uniform nanoparticles with an average particle size of approximately 220 nm, and a negatively charged particle surface (Fig. 2).



Diameter (nm)	219.3 ± 4.0
Polydispersity Index	0.092 ± 0.001
Zeta-potential (mV)	-13.2 ± 1.0
methemoglobin conc. (%)	> 95
Remaining NO_2^- (%)	< 0.16

Figure 2. Structure of metHb@Lipo
metHb@Lipo exhibits a nanoparticle structure in which high concentrations of methemoglobin are encapsulated within a lipid bilayer.

Next, in order to verify the cyanide binding affinity of metHb@Lipo, researchers measured the shift of absorption spectra when sodium cyanide was added to metHb@Lipo. Consequently, after adding sodium cyanide, the spectra shifted rapidly from the absorption spectrum derived from metHb@Lipo to the absorption spectrum derived from cyanide-bound metHb@Lipo. Furthermore, when the total cyanide concentration in the blood of mice administered with sodium cyanide was evaluated, cyanide was retained in the blood at a higher rate when metHb@Lipo was administered than in the group administered with saline. These results suggested that by binding to cyanide, metHb@Lipo pulled cyanide that had transferred to cytochrome c oxidase in tissue mitochondria back into the blood, or rather that it prevented cyanide in the blood from transferring to cytochrome c oxidase in tissue mitochondria (Fig. 3).

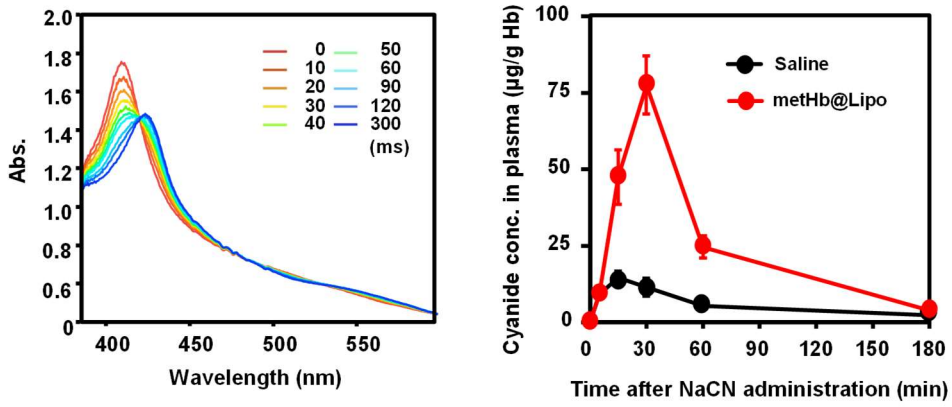


Figure 3. Cyanide binding affinity of metHb@Lipo
metHb@Lipo rapidly binds to cyanide *in vitro* (left) and *in vivo* (right). NaCN: sodium cyanide

To assess the detoxification effect of metHb@Lipo on cyanide poisoning, mice administered with lethal doses of sodium cyanide were treated 10 minutes later with metHb@Lipo therapy, a sodium nitrite monotherapy, or a sodium nitrite and thiosulfate combination therapy, and the survival was compared. The results showed higher survival in mouse models for cyanide poisoning treated with metHb@Lipo therapy compared to the existing cyanide poisoning antidotes of sodium nitrite monotherapy and sodium nitrite and thiosulfate combination therapy. The study also revealed that the mice treated with metHb@Lipo were quicker to recover from comas, demonstrating the fast-action of the treatment (Fig. 4).

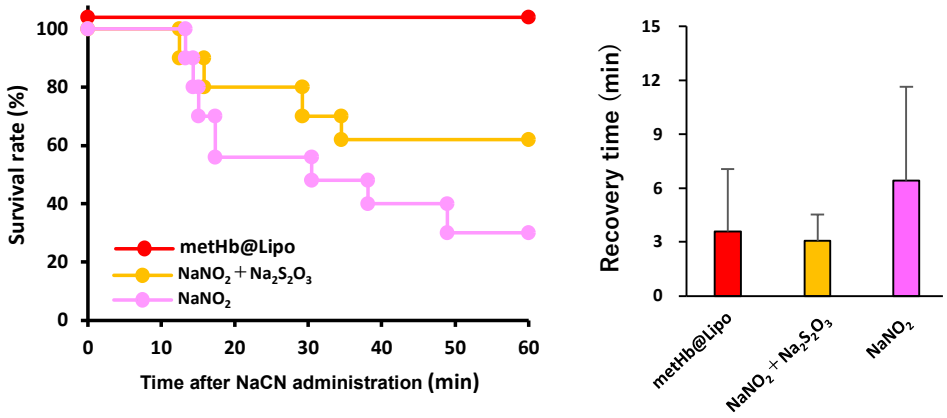


Figure 4. Evaluation of the detoxifying effect of metHb@Lipo in mice with lethal cyanide poisoning

Administration of metHb@Lipo to mouse models for lethal cyanide poisoning showed higher survival compared to existing drug treatment groups (sodium nitrite monotherapy, sodium nitrite and sodium thiosulfate combination therapy) (left). Additionally, a trend showing rapid recovery emerged after evaluating the revival periods of the surviving mice (right).

NaCN: sodium cyanide, $NaNO_2$: sodium nitrite, $Na_2S_2O_3$: thiosulfate

When the cytochrome c oxidase activity in mouse models for cyanide poisoning treated with metHb@Lipo therapy or sodium nitrite and thiosulfate combination therapy were measured over time to assess cytochrome c oxidase in the mitochondria (the cause of cyanide poisoning), it was found that metHb@Lipo therapy rapidly restored activity that had been diminished by cyanide administration. Additionally, acidosis induced by tissue hypoxia, which is a symptom of cyanide poisoning, also showed rapid recovery after treatment with metHb@Lipo (Fig. 5).

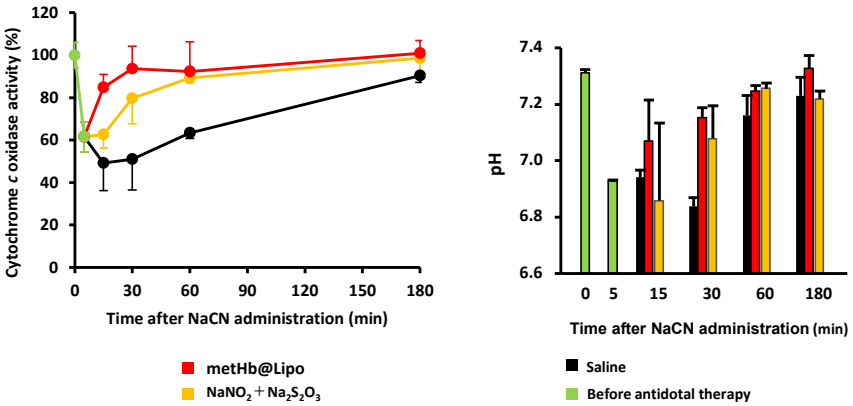


Figure 5. Detoxification mechanism of metHb@Lipo in mice with cyanide poisoning

Administration of metHb@Lipo to mice with cyanide poisoning rapidly restored cytochrome c oxidase activity compared to the existing drug treatment group (sodium nitrite monotherapy, sodium nitrite and sodium thiosulfate combination therapy) (left). It also rapidly restored the decrease in blood pH (acidosis) induced by cyanide poisoning (right).

NaCN: sodium cyanide, $NaNO_2$: sodium nitrite, $Na_2S_2O_3$: thiosulfate

4. Conclusion

From this research, it was demonstrated that metHb@Lipo has a stronger and faster detoxification effect on cyanide poisoning than existing nitrite preparations. Furthermore, because administration of metHb@Lipo does not inhibit oxygen transport in red blood cells, tissue hypoxia also showed rapid improvement (improvement of acidosis). Thus, metHb@Lipo can be used even when cyanide poisoning occurs in situations where use of nitrites is not possible (such as smoke inhalation from fires), and its clinical use as a novel antidote to cyanide poisoning is expected to expand treatment options moving forward.

5. Details of Original Paper

Title: Liposome-encapsulated methemoglobin as an antidote against cyanide poisoning
Authors: Yuto Suzuki, Kazuaki Taguchi, Tomoko Kure, Hiromi Sakai, Yuki Enoki, Masaki Otagiri and Kazuaki Matsumoto
Journal: *Journal of Controlled Release (online edition)*
DOI: 10.1016/j.jconrel.2021.07.015

Glossary

(Note 1) Liposome preparation: Liposome preparations are a technology developed for the purpose of efficiently delivering a drug to a target site. They are a formulation that consists of a drug product that is encapsulated with lipid membrane that mimic the composition of biological membranes such as cell membranes. Due to this structure, liposomes allow for improved stability of drug formulations in the bloodstream of a living organism.

(Note 2) Methemoglobin: Methemoglobin is a trivalent iron state obtained by oxidizing the heme iron contained in hemoglobin in red blood cells from a divalent state. In the divalent state of heme iron, it binds to oxygen and can transport it to tissue, but in the trivalent state methemoglobin loses its oxygen-carrying capacity.

(Note 3) Nitrites (nitrous acid compounds): Nitrites are substances used as color-producing reagents for ham and sausage, etc. As nitrites also have an oxidizing effect, they are used as an agent for oxidizing hemoglobin in red blood cells to detoxify cyanide poisoning.

(Note 4) Conversion of hemoglobin to methemoglobin: Heme iron that composes hemoglobin is oxidized from divalent to trivalent iron ions.

(Note 5) Hemoglobin vesicles: Hemoglobin vesicles are an artificial red blood cell preparation. Hemoglobin extracted from outdated human red blood cells are encapsulated in a

phospholipid bilayer membrane. Hemoglobin vesicles are expected to play a role in addressing important upcoming medical issues such as blood transfusion supply shortages due to the declining birthrate and aging population.

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