Development of platinum nanoparticles, liver-targeting reactive oxygen species scavenger, for prevention of hepatic ischemia/reperfusion injury

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ABSTRACT SUMMARY

Reactive oxygen species (ROS) are involved in the pathophysiology of ischemia/reperfusion injury. To protect hepatocytes from ischemia/reperfusion injury in mice, we developed platinum nanoparticles, ROS scavenger possessing the ability to target a specific type of liver cells. Platinum nanoparticles effectively scavenged hydrogen peroxide and other ROS in buffer solutions. Platinum nanoparticles predominantly accumulated in the liver after intravenous injection in mice. In an ischemia/reperfusion injury mouse model, in which hepatic injury was induced by occluding the portal vein for 15 min followed by a 6 h reperfusion, the elevation of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities was significantly inhibited by a bolus intravenous injection of platinum nanoparticles. These results indicate that platinum nanoparticles can be used to prevent hepatic ischemia/reperfusion injury.

INTRODUCTION

Oxidative stress has been widely recognized to be involved in the pathogenesis of cardiopulmonary disorders and ischemia/reperfusion injury. Therefore, ROS scavengers have been considered as therapeutic agents for ROS-mediated injuries and diseases. The use of L-cysteine and tocopherol, representative ROS scavengers, has been examined for various ROS-mediated injuries, especially those associated with ischemia/reperfusion. However, these existing ROS scavengers easily pass through biological membranes and diffuse freely throughout the body due to their small size, which limits their therapeutic potential. Therefore, novel ROS scavengers with better pharmacological and pharmacokinetic properties are highly required to improve the cytoprotective activity of ROS scavengers.

Of various possible strategies, micro- or nanoization of ROS scavengers appears to be a good approach to controlling their pharmacokinetic properties, because micro-and nanoparticles tend to accumulate in the macrophage where ROS are generated in ischemia/reperfusion, i.e., hepatic Kupffer cells.

The aim of this study is to develop a novel ROS scavenger possessing the ability to target hepatic Kupffer cells for prevention of hepatic ischemia/reperfusion injury. To this end, we fabricated platinum nanoparticles, liver-targeting ROS scavenger, by reacting platinum hydrochloride ion with reductant. Then, the scavenging effect of ROS in a buffer solution and tissue distribution after intravenous injection in mice were examined. Finally, the therapeutic potential of platinum nanoparticles was investigated in a hepatic ischemia/reperfusion injury model in mice.

EXPERIMENTAL METHODS

Platinum nanoparticles were fabricated by reacting platinum hydrochloride ion with reductant. The particle size and zeta potential of platinum nanoparticles were determined using a Zetasizer Nano ZS instrument.

Scavenging effects of platinum nanoparticles on ROS were evaluated by a fluorescent probe method.

The tissue distribution after intravenous injection of platinum nanoparticles in mice was determined using ICP-MS.

In the ischemia/reperfusion injury model, in which hepatic injury was induced by occlusion of the portal vein and the hepatic artery for 15 min followed by 6 h reperfusion under pentobarbital anesthesia, the elevation of plasma ALT and AST activities was assayed as indicators of hepatocyte injury. The lipid peroxide in the liver was also measured as an indicator of oxidative stress. Platinum nanoparticles (50 µg platinum/kg) were given via the tail vein just before reperfusion was initiated.

All animal experiments were conducted in accordance with the principles and procedures outlined in the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The protocols for animal experiments...
were approved by the Animal Experimentation Committee of the Kyoto Pharmaceutical University.

RESULTS AND DISCUSSION
Platinum nanoparticles were approximately 20 nm in diameter and slight negative zeta potential (Figure 1). This nanoparticles effectively scavenged ROS, including hydrogen peroxide and hydroxyl radical. Platinum nanoparticles rapidly disappeared from the blood, and approximately 80% of the platinum nanoparticles accumulated in the liver by 10 min after their intravenous injection in mice (Figure 2).

Furthermore, platinum nanoparticles predominantly accumulated in the liver nonparenchymal cells (NPC), including Kupffer cells and endothelial cells, after their intravenous injection in mice. The ischemia followed by reperfusion resulted in a striking increase in plasma ALT and AST activities. Platinum nanoparticles effectively suppressed elevation of the markers (Figure 3). The elevation of lipid peroxide content in the liver was significantly inhibited by a bolus intravenous injection of the platinum nanoparticles, just before the start of reperfusion. These results suggested that platinum nanoparticles protected hepatocytes from ischemia/reperfusion injury through the scavenging ROS.

Figure 3. Effect of platinum nanoparticles on plasma AST and ALT levels in mice following 6 h of reperfusion. (a) naïve; (b) saline; (c) platinum nanoparticles (50 µg platinum/kg). □, ALT; ■, AST. *p < 0.05: significantly different from the saline treated group.

CONCLUSION
We have successfully developed platinum nanoparticles, novel ROS scavenger, which exhibited better ROS scavenging effect and selective accumulation to the NPC where ROS were generated in hepatic ischemia/reperfusion. Platinum nanoparticles effectively prevent hepatic injury after ischemia/reperfusion. These findings indicate that platinum nanoparticles are promising material for preventing hepatic ischemia/reperfusion injury.

REFERENCES