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ORIGINAL ARTICLE

Nanoparticle-delivery system enhanced the improvement and recovery in toxicity-induced acute hepatic failure

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Abstract

Background: The major curative remedy for advanced liver failure is hepatic transplantation. However, the conventional medicine still shows the limitations and obstacles for liver regeneration. Importantly, it is unclear whether we can get a rapid and high efficacy platform to facilitate to reprogram hepatic capability. The main work of this study was to develop a platform for a nanomedicine-based gene-delivery platform of novel nanoparticles (NNPs) to efficiently facilitate the liver function recovery.

Methods: In this study, we studied the feasibility and efficiency of NNP and produced the multiple abilities of NNPs for a potential platform of gene transduction. We showed that NNPs played an important role in hepatic protection. The cytoprotective effects of NNPs in toxic-hepatic cells were investigated and evaluated by cell viability, reactive oxygen species production, in vitro cell abilities, and in vivo animal studies.

Results: We demonstrated that NNPs possess the abilities to protect the cell after toxic-stress both in vitro and in vivo. Under the stress condition, our result showed that cell viabilities can be improved by NNP-carried hepatocyte nuclear factor 3 (*HNF3*) gene (NNP-*HNF3*), which is a famous hepatic transcriptional factor and regenerative marker to modulate essential molecular pathways activating various hepatic-specific markers. Importantly, compared to control and NNP-control, *NNP-HNF3* exhibited the cytoprotective effects that prevented toxic-induced oxidative stress and cell damage in vitro as well as in vivo. Notably, our data showed that *NNP-HNF3* treatment may improve toxic-induced hepatic encephalopathy.

Conclusion: Herein, we demonstrated that novel nanoparticle, such as *NNP-HNF3*, serves as a key regulator for protecting the damaged hepatic cell and the bioproduct-based source for the new therapeutics of hepatic failure.

Keywords: Cytoprotective effect; Hepatic failure; HNF3; Novel nanoparticle

1. INTRODUCTION

Acute hepatic failure (AHF) is a fulminant and complicated hepatic damage with organ failure, elevated hepatic enzymes, and finally causes hepatic encephalopathy. It is a big challenge to treat severe AHF in medical treament.[1,2] Although liver transplantation provides a curative treatment for hepatic failure, there are still many limitations for hepatic transplant treatment.[2–6] Stem cell therapeutics are the promising way for treating hepatic diseases.[7–9] Pluripotent stem cells including embryonic stem cells and induced pluripotent stem cells can produce mature liver cells.[10–13] However, limitation of good biosources and biocellular products for delivering the targets poses the major difficulty for treating the damaged liver and further facilitating hepatic regeneration.

Nonviral gene delivery, such as the use of biodegradable nanoparticles, has been considered a potentially safer gene-delivery method in comparison to conventional virus-based systems.[14–17] The multi-step processing including polycationization of DNA-complexities could protect DNA degradation, and these DNA complexes are small enough to facilitate the cells maturing into intracellular organs.[15–19] The novel particles, polymers, and polycomplex can significantly and efficiently bind to the cell membrane and intracellular organs.[16–19] Cationic nanoparticles have been shown to be noncytotoxic and are characterized by high transfection efficiency, which makes them a promising in vivo gene-delivery material for disease treatment. It has been reported that cationic nanoparticle-mediated delivery of miR-145 and Oct4 with Sirt1 in mice suppressed lung adenocarcinoma tumor progression and promoted functional recovery of aged retinas, respectively.[18]

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Hepatocyte nuclear factor 3 (HNF3), a critical hepatic transcriptional factor, serves a pivotal role in metabolism regulation and in the hepatic metabolism. In this report, we developed a method for reconstruction of damaged hepatic cells by applying the nanoparticle-delivery system. We developed a method to efficiently deliver a hepatic-specific factor that can improve cell therapy and tissue engineering in the treatment in an AHF model. We herein investigated the hepatoprotective function of novel nanoparticles (NNPs) in a carbon tetrachloride (CCl_4), a severe toxic substance causing liver damage, induced AHF mouse platform. Our findings revealed that NNPs could effectively protect the injured hepatic organs and significantly improve the outcomes in mice pretreated with CCl_4. In this study, we demonstrated that this novel NNP system would provide a new way for medical research and develop a drug-driven strategy for recovering hepatic disorders.

2. METHODS

2.1. Synthesis of NNPs

The study materials, biosynthetic products, and chemicals were delivered from commercial companies. NNPs were obtained and synthesized by modifying N-trimethoxysilylpropyl-N,N,N-trimethylammonium chloride (TA). All used chemicals were without further purification.

2.2. Real-time reverse transcription-polymerase chain reaction

In this study our protocols are as follows: the RNA was isolated from cells using Trizol reagent and the QiagenRNAeasy (Qiagen, USA) column was used for purification, according to the manufacturer's instructions. The RNA was extracted and quantized by using UltroSpec 3100 Pro, and 1 μg of RNA was reversely transcribed with a SuperScript III reverse transcriptase kit (Invitrogen, USA). All cDNA amplification was done in a total volume of 15 mL containing 0.5 mM of each primer, 6 mM $MgCl_2$, 3 mL LightCycler FastStart DNA Master SYBR green I (Roche Diagnostics, Pleasanton, CA), and 1.5 mL of 1:12 diluted cDNA, and then evaluated by using LightCycler Roche Diagnostics. *GAPDH* housekeeping gene was amplified as a reference standard in each experiment. Moreover, the final steps, including PCR-reactions, were done in triplicate and then heated to 95°C for 10 minutes, as done by 35 cycles of denaturation at 95°C for 10 seconds, and further annealing at 60°C for 5 seconds, and the extension of final product at 72°C for 20 seconds.

2.3. Cell viability assay

HepG2 and PLC were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA). The cell viability and proliferation ability of HepG2 and PLC was measured by MTT test and other assays. In brief, NNPs were seeded on 24-well plates at a density of 2 × 104 cells/well in medium and submitted to MTT assay (Sigma-Aldrich). Cells were incubated with 0.25 g/L MTT for 4 hours at 37°C, and added with 100% isopropanol to terminate the reaction. The MTT formazan product was measured by using a microplate reader and the optical density (OD) at 570 nm were detected (SpectraMax 250, Molecular Devices, CA, USA). The cell viability was measured using the OD values.

2.4. Animal model of liver injury

The animal study has been approved by TVGH Animal Committee, and the principles of Laboratory Animal Care were performed. Eight-weeks-old mice (25 to 30 g, male B6 mice) were used for our experiments. Intraperitoneal injection of CCl_4 (2.5 mL/kg body weight) was given to induce fulminant hepatic failure. Six hours after the administration of CCl_4, B6 mice were intraperitoneally injected with PBS, NP, or NP-HNF3 at four different dosages. After the administration of CCl_4, the evaluation was performed at 24, 48, or 72 hours in recipients of PBS, NP, or NP-HNF3 to monitor hepatic damage.

2.5. Determination of intracellular reactive oxygen species production and nitrate/nitrite concentration

Cells were incubated with 5 μmol/L of 2′,7′-dichlorofluorescein diacetate (DCFH-DA) in a culture medium for 30 minutes at 37°C, and then flow cytometry analysis was performed to measure intracellular reactive oxygen species (ROS) production by the probe DCFH-DA (Molecular Probes, Eugene, USA). Total nitrite in liver tissue was assayed by adding 100 μL Griess reagent (0.05% naphthalethylenediamine dihydrochloride and 0.5% sulphanilamide in 2.5% phosphoric acid) to each sample. The total nitrite/nitrate concentration of the sample was measured at OD 550 nm in comparison with a standard solution of sodium nitrate prepared in saline.

2.6. Malondialdehyde assay

The measurement of malondialdehyde (MDA) in liver tissue was performed by using an enzyme-linked immunosorbent assay (ELISA) reader (Bio-Rad Laboratories, Hercules, CA, USA) by determining absorbance at 530 nm.

2.7. Statistical analysis

In this study, we used the statistical analysis as mean ± SD. Statistical analysis was performed using Student's *t* test or a one-way or two-way analysis of variance test followed by Turkey's test, as appropriate. $p < 0.05$ was considered to be statistically significant.

3. RESULTS

3.1. Characterization and preparation of NNPs

In this study, we attempted to develop NNPs for the efficient gene delivery of *HNF3* and examined NNP-based delivery of HNF3 could rescue acute severe hepatic failure. The uniform nanoparticles were prepared under proper ratio. Briefly, tetraethyl orthosilicate and cetyltrimethylammonium bromide (surfactant) were prepared and used in this study. The NNPs were characterized by FT-IR and ^{1}H NMR (400 MHz, $DMSOd_6$, ppm; data not shown) spectroscopies. NNP-sbPEI was synthesized using the aminolysis reaction of polyurethane (c) and small branch polyethyleimine (MW = 600) (sbPEI). The average size of NNPs is 100 nm. The ultramicroscopic structure was shown in Figure 1A, B.

3.2. NNP-mediated delivery of HNF3 into hepatoma cells

In this study, we used the NNPs, a noncytotoxic vehicle, which are capable of high transfection activity in a delivery vehicle. After the generation and characterization of NNPs, stable HNF3-overexpressing hepatic cells were quickly generated using the NNP-delivery system (Fig. 1C) with plasmid vectors. The pHNF3 was loaded on NNPs complexes by electrostatic adsorption. We further explored the optimal concentration and mixture ratio for HNF3cDNA with the NNPs. Notably, our results demonstrated that the ratio of pHNF3β to NNP is fixed on 1:32 for the gene-delivery studies. To further measure the roles of *NNP-HNF3* gene transduction in hepatic cells in vitro, an empty vector-delivery control and NNP-HNF3 was produced simultaneously and transfected into hepatic cell lines (Fig. 2A). The transfection of HNF3 into HepG2 cells is shown in Figure 2A. Moreover, our data showed that the CCl_4-mediated toxicity could further damage the liver cell in vitro. Importantly, the results of cell proliferation and viability assay showed that the treatment of NNP-HNF3 may protect the CCl_4-damaged hepatic cell (HepG2 and PLC cells in Fig. 2B).

3.3. Amelioration of fulminant hepatic failure by transplantation of NNP-HNF3-treated hepatocytes

Furthermore, we examined whether NPP-HNF3 could improve acute liver damage and hepatic failure in CCl_4-treated mouse with acute liver injury. To detect acute injuries, a dosage of CCl_4-2.5 mL/kg was administrated intraperitoneally. B6 mice

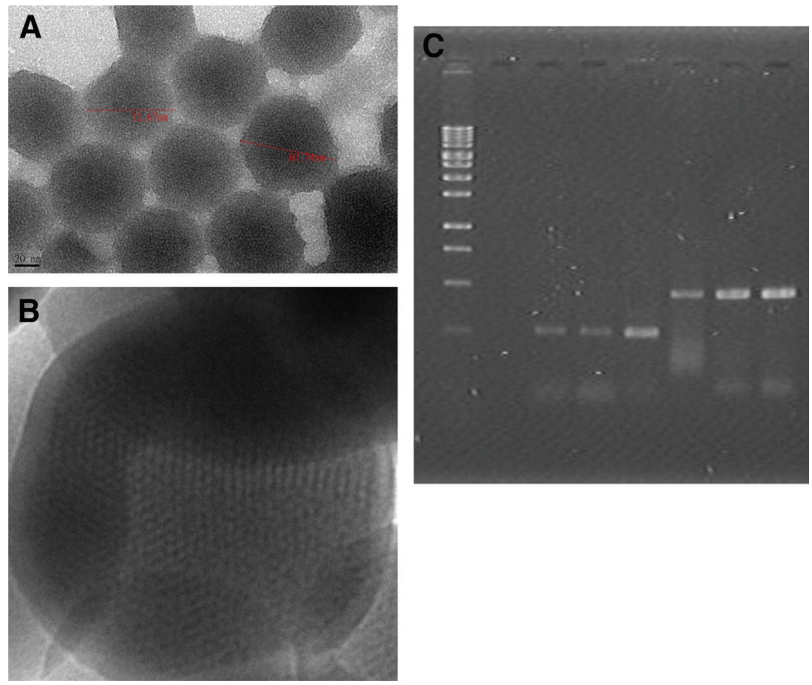

Fig. 1 Preparation and characterization of NNPs. A, The polyurethane was precipitated and purified in ethyl ether and dried at 40ºC under vacuum. The NNPs were characterized by FT-IR and ^{1}H NMR (^{1}H-NMR (400 MHz, $DMSOd_6$, ppm). NNP-sbPEI was synthesized using the aminolysis reaction of polyurethane and sbPEI (MW = 600) in scheme 1. B, The pore size distribution was calculated by BJH method following the detection of image intracellular distribution of nanoparticles. Cells were fixed with TEM fixation solution for another 24 hours. C, To test this hypothesis, the stable HNF3-overexpressing hepatic cells were quickly generated using the NNP-delivery system with plasmid vectors. The pHNF3 was loaded on NNPs complexes by electrostatic adsorption. When the ratio of pHNF3 to NNP was < 1:32, a stable complex was formed with no free pHNF3 detected by using agarose gel electrophoresis. HNF3, hepatocyte nuclear factor 3; NNP, novel nanoparticles; sbPEI, small branch PEI.

were delivered into recipients with either PBS or NNP. The effect of NNP-HNF3 was not obvious among all given NNPs with or without HNF3 plasmids, such as 2×10^5, 5×10^5, 2×10^6, and 5×10^6/kg. NNP alone showed no additional effect on the cell therapy, whereas transplantation of NNP-HNF3 in CCl_4-treated mice led to a remarkable reduction in necrotic area in CCl_4-induced AHF (Fig. 3A). We next investigated that the treatment by injecting NNP-HNF3 in CCl_4-treated mice reduced the production of oxidative stress in vivo. MDA and nitrate/nitrite are indicative of oxidative damage. Furthermore, using

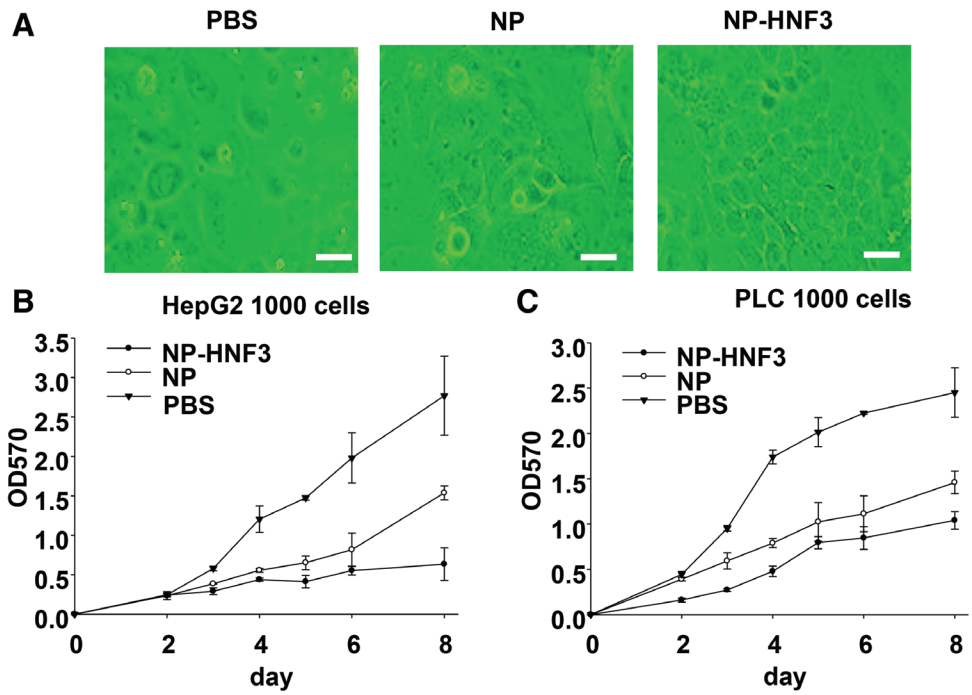

Fig. 2 Characterization of *NNP-HNF3* gene transduction in hepatic cells. A, To further measure the roles of *NNP-HNF3* gene transduction in hepatic cells in vitro, an empty vector-delivery control and NNP-HNF3 was produced simultaneously and transfected into hepatic cell lines. B, To further screen and examine the hepatoprotective activity of NNPs in CCl_4-treated HepG2 cells and (C) PLC cells. B and C, Our data support NNP-HNP3 could effectively improve the cell viabilities and cell-cycle-based proliferation in treated HepG2 (B) and PLC hepatic cells (C). In addition, we evaluated whether NNP and NNP-HNF3 were resistant to CCl_4-induced cell death (Bar: 100 μm). *$p < 0.05$. CCl_4, carbon tetrachloride; HNF3, hepatocyte nuclear factor 3.

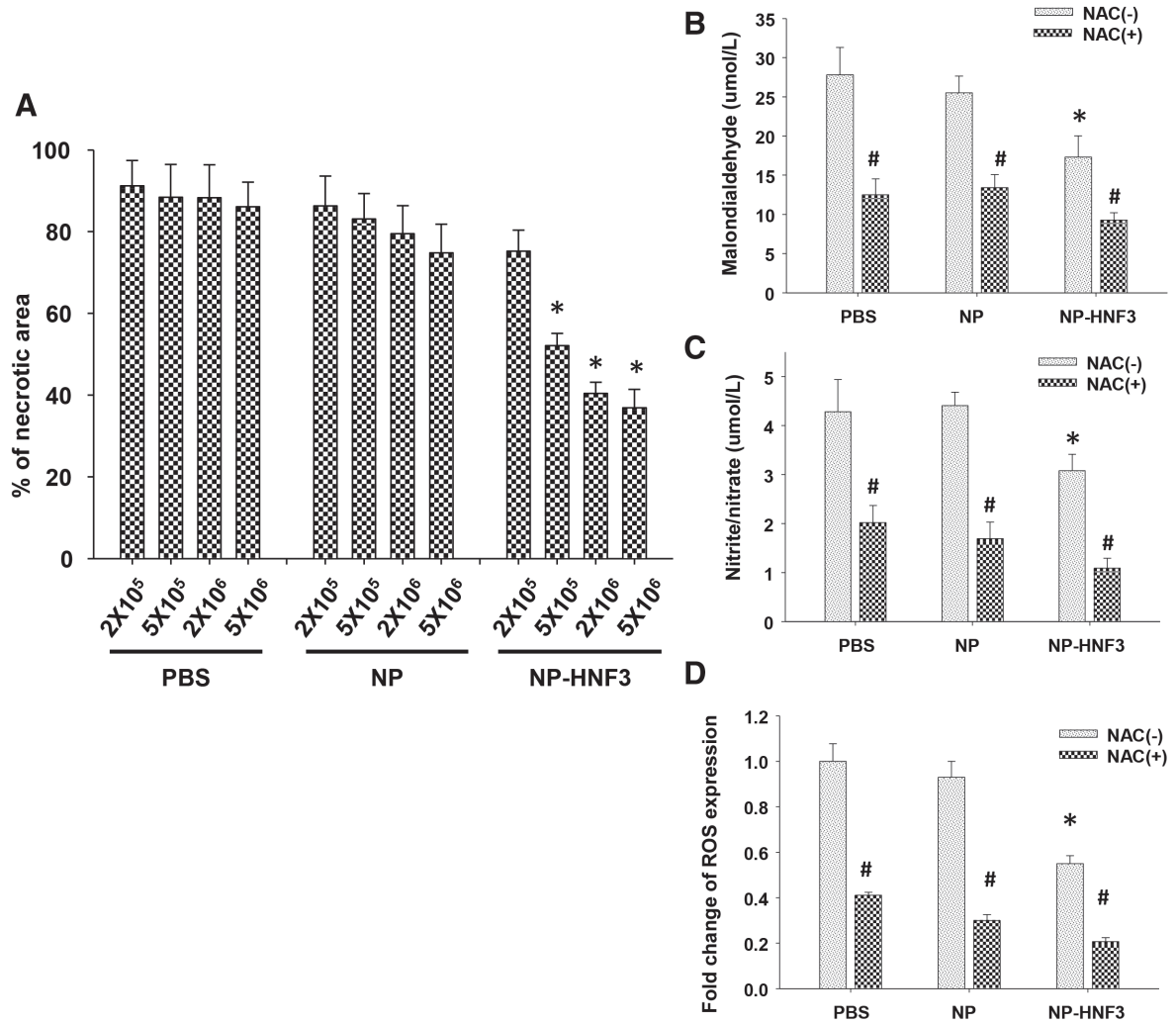

Fig. 3 *NNP-HNF3* gene transduction suppressed the ROS production in damaged hepatic regions. A, The delivery efficacy and effects of NNP-HNF3 was not obvious at doses 2×10^5, 5×10^5, 2×10^6, and 5×10^6/kg, whereas transplantation of either NNPs led to a remarkable reduction in necrotic area in CCl_4-induced AHF. B–D, The hepatoprotective effect of NN-HNF3 was dose-dependent and was higher than that of NNP only and PBS controls. The studies including MDA and nitrate/nitrite are indicative of oxidative damage. CCl_4 treatment induced the production of MDA, nitrate/nitrite, and ROS in livers from all recipients. The CCl_4-induced production of MDA, nitrate/nitrite, and ROS and significantly suppressed NNP-HNF3 treatment compared to NNP only and PBS control. In panel B–D, $^*p < 0.05$ vs PBS or NP, #$p < 0.05$ vs corresponding to recipient with NAC treatment. AHF, acute hepatic failure; CCl_4, carbon tetrachloride; NAC, N-acetyl-cysteine; ROS, reactive oxygen species.

the toxic model such as CCl_4 administration elicited the production of MDA, nitrate/nitrite, and ROS in livers from all treated-groups in CCl_4-treated mice (Figure 3B–D). The CCl_4-induced production of MDA, nitrate/nitrite, and ROS were not affected by NNP-HNF3 transplantation (Figure 3B–D). Moreover, the CCl_4-induced production of MDA, nitrate/nitrite, and ROS significantly reduced in livers from recipients of NNP-HNF3-based transplantation. N-acetyl-cysteine (NAC), an antioxidant, can minimize ROS. Administration of NAC decreased the CCl_4-induced damages in causing the oxidative ROS and toxicity in the different groups with NNP-HNF3 or control treatment. The maximal inhibition of oxidative substances was observed in recipients of NNP-HNF3-based therapeutics. In addition, the mobility and capabilities of motor activity studies are done. After treated with CCl_4 for seventy-two hours, the result showed that the total mobility and ambulatory movements could be significantly increased and improved in groups of NNP-HNF3, compared to all other groups (Fig. 4).

4. DISCUSSION

Herein we discovered that NNP-HNF3 exhibited a prominent antioxidant system both in vitro and in vivo. For ensuring the hepatoprotective property of NNPs in vivo, we tested the cytoprotection of NNPs after cell transplantation into the mouse model of AHF, induced by the hepatotoxic CCl_4. Importantly, the gene delivery of NNPs-HNF3 effectively rescued these CCl_4-treated mice. Our key results further presented to significantly decrease liver damage area and oxidative substances with recovering hepatic functions and mobility activities.

Previous studies have showed that hepatic encephalopathy causes high mortality rate, occurrence of confusion, as well as changing level of consciousness and coma.[20–23] This is the major complication associated with fulminant hepatic failure. This study showed that NNP could effectively improve AHF. Using this NNP gene-delivery system, we can provide a potential platform of NNP-HNF3 presenting therapeutic effect against hepatic encephalopathy. Our findings have showed the evidence to demonstrate NNP-HNF3 treatment could not only protect liver damage but also effectively improve toxic-induced hepatic encephalopathy.

In conclusions our findings showed that NNPs effectively rescued CCl_4-induced AHF, and the hepatoprotective effect of NNP-HNF3 could present the potential roles in modulating hepatoprotective effects to repair injured tissues. In the future, it will be a potential platform to take the advantage of the hepatic

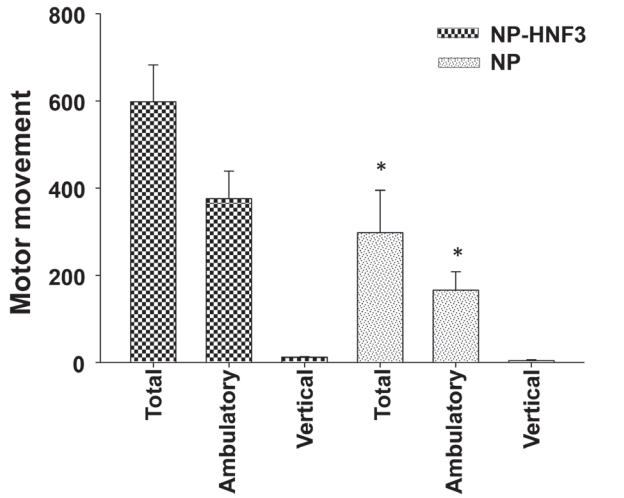

Fig. 4 *NNP-HNF3* gene transduction improved the motor function in AHF mice model. The assay of motor activity was monitored at 72 h after CCl_4 administration; the total movements and ambulatory movements were observed in NNP-HNF3 recipients compared to mice, which had received NNP only and PBS injection only (each group: n = 3); *$p < 0.05$. AHF, acute hepatic failure; CCl_4, carbon tetrachloride; HNF3, hepatocyte nuclear factor 3; NNP, novel nanoparticles.

protection function and efficacy of the NNP-HNF3 in fulminant liver failure. In addition, the NNP system carrying the gene products, small molecules, and pharmaceutic peptides may serve as an alternative to therapeutics to treat hepatic failure.

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