A novel bone marrow targeted gadofullerene agent protect against oxidative injury in chemotherapy

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ABSTRACT Chemotherapy as an effective cancer treatment technique has been widely used in tumor therapy. However, it is still a challenge to overcome the serious side effects of chemotherapy, especially for its myelotoxicity. Here we report a novel strategy using the water soluble gadofullerene nanocrystals (GFNCs) to protect against chemotherapy injury in hepatocarcinoma bearing mice, which was induced by the commonly chemotherapeutic agent cyclophosphamide (CTX). The GFNCs were revealed to specifically accumulate in the bone marrow after intravenously injecting to mice and they exhibited excellent radical scavenging function, resulting in a prominent increase of mice blood cells and pathological improvements of the primary organs in the GFNCs (15 mg kg⁻¹) treated mice after the CTX (60 mg kg⁻¹) therapy. Moreover, the GFNCs maintained and even strengthened the anti-neoplastic activity of the CTX agent. Therefore, the GFNCs would be the promising chemoprotective agents in chemotherapy based on their high efficiency, low toxicity and metabolizable property.

Keywords: gadofullerene nanocrystals, chemopreventive agent, myelosuppression, radical scavenging, chemotherapy drug

INTRODUCTION

Though many tumor-targeted therapies have been developed in recent years, cancer is still one of the most fatal diseases in the world, and chemotherapy is still the most popular cancer treatment technique due to its convenience and high efficiency [1,2]. However, most chemotherapeutic drugs kill the tumor cells and those proliferating cells non-selectively [3,4], so it often leads to serious injury to normal tissues [5–9]. For example, chemotherapy generates a large amount of excess reactive oxygen species (ROS) in the bone marrow that would result in a random

damage to the hematopoietic tissues [10,11], leading to serious myelosuppression [12–14] as well as severe injury of some normal organs [15,16].

In order to overcome the side effects of chemotherapy, researchers have developed many techniques to protect normal tissues against the chemotherapy injury, e.g., performing a cancer cell targeting therapy to avoid acting upon normal cells, or simply administering some chemoprotective drugs [17,18] such as antioxidants [19–21] and leukopoietic agents [22–24] in the chemotherapeutic process. But the chemoprotective drugs usually lead to some new problems, for example, the clinic leukopoietic agents may recombine human granulocyte-colony stimulating factor (rhG-CSF), which not only leads to serious bone pain of patients, but also causes the bone marrow hematopoietic cells to become fatigue.

Along with the rapid development of nanotechnology, various functional nanomaterials have shown great potential in biomedicine applications [25–27]. Of them we have special interests on fullerenes and metallofullerenes due to their low toxic [28–30] metabolizable properties *in vivo* [31–33], as well as their excellent radical scavenging properties. In fact, fullerenes have been reported to protect cells from both ROS hurt and regulate apoptotic process in different biological model systems [34–38], e.g., Moussa *et al.* [39] recently reported that oral administration of C₆₀ dissolved in olive oil could reduce the oxidative stress induced by the CCl₄ intoxication and largely prolong the lifespan of test rats to double.

Besides of empty fullerenes, we recently reported a new powerful tumor vascular-targeting therapeutic technique using gadofullerene nanocrystals (GFNCs) for the solid cancer therapy [32]. It was revealed that the GFNCs have a high-level accumulation in the bone of mice after in-

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travenous injection, which was in accordance with previous investigations [31,40]. Therefore, based on the fact that GFNCs are excellent radical scavengers and most chemotherapeutic agents induce myelosuppression through radical damaging way, it is natural to expect the GFNCs would be an excellent candidate as chemoprotective agent in tumor therapy. In this work, by using the clinical cyclophosphamide (CTX) as chemotherapeutic agent and GFNCs as protective agents, we elaborately designed and performed a series of cell experiments and animal tests, and the results strongly indicate that GFNCs can selectively enter the bone marrow and show significant chemoprotective effects against CTX-induced toxicity in mice. Moreover, this material is metabolizable and low toxic to normal tissues, so it is highly potential to realize clinical applications in future.

EXPERIMENTAL SECTION

Preparation of GFNCs

Water-soluble GFNCs were prepared through a simple reaction according to previous report [32]. Simply, a suspension of 100 mg Gd@C_{82} (99.5% purity, as obtained from Xiamen Funano Co.) in 35% aqueous H_2O_2 (7 mL) and 12% NaOH (3 mL) was stirred vigorously at 50°C under ambient conditions for 2 h. The black suspension gradually dissolved, yielding a yellow solution with slight insoluble particles. A transparent yellowish solution was obtained after filtration. The solution was transferred to a dialysis bag to remove impurities and Arsenazo III was added to the concentrated dialyzate to confirm that there was no residual dissociative Gd^{3+} . The obtained yellowish powder was purified with Sephadex G-25 gel filtration medium with Milli-Q water as the eluent. Then, the hydroxyl-bearing water-soluble GFNCs were lyophilized for later use.

Characterization of GFNCs

The hydrodynamic size of GFNCs was characterized by dynamic light scattering (DLS, Malvern Zetasizer Nano ZS 90) at 25°C. Atomic force microscopy (AFM) images were obtained by JEM-2011 (JEOL, Japan) operating at 200 kV for characterizing the size and thickness of GFNCs.

Hydroxyl radical scavenging activity measurements *in vitro*

The *in vitro* hydroxyl radical-scavenging capability of the GFNCs was examined by electron spin-resonance (ESR) spectroscopy (Bruker ELEXSYSE ESR spectrometer, German) *via* spin-trapping using 5,5-dimethyl-1-pyrroline-*N*-

oxide (DMPO) as a trapping agent. Hydroxyl radicals were generated by ultraviolet (UV)-irradiation for 8 min with 150 μL of reaction mixture containing 0.133 mol L^{-1} phosphate buffered saline (PBS) and 0.133 mmol L^{-1} DMPO. Subsequently, 50 μL of GFNCs solution with a Gd^{3+} concentration of 50 $\mu\text{mol L}^{-1}$ was added to the above solutions. The ESR data were collected at 3 min after initiating the formation of hydroxyl radicals by the addition of H_2O_2 at room temperature.

Cell culture

FDC-P1 cells (A kind of mice bone marrow cells, purchased from Shanghai cell bank, type culture collection committee, Chinese Academy of Sciences) were used to investigate the cell protective effects of GFNCs. FDC-P1 cells were cultured with Dulbecco minimum essential medium (DMEM, Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone Company, South Logan, UT), penicillin (100 $\mu\text{g mL}^{-1}$), streptomycin (100 $\mu\text{g mL}^{-1}$), and interleukin-3 (IL-3, 0.002 $\mu\text{mol L}^{-1}$) (Gibco, Grand Island, NY, USA) in a humidified incubator with 5% CO_2 at 37°C.

Cytotoxicity of GFNCs towards FDC-P1 cells

For the cytotoxicity examination, about 1×10^4 FDC-P1 cells were incubated with GFNCs (Gd^{3+} concentrations of 0–30 $\mu\text{mol L}^{-1}$) in the dark at 37°C for 24 h. The cell viability was evaluated using a Cell Counting Kit-8 (CCK-8; DOJINDO, Kumamoto, Japan) assay. The absorption values at 450 nm were detected by a 96-well plate reader (iMark microplate reader, Bio-RAD, USA) to determine the viability ($\text{cell viability} = (\text{OD}_{\text{tre}} - \text{OD}_{\text{medium}}) / (\text{OD}_{\text{con}} - \text{OD}_{\text{medium}})$), where OD_{tre} is the absorption value of treated cells, OD_{con} is that of the control cells, and $\text{OD}_{\text{medium}}$ is that of the culture medium.

Hydroxyl radical scavenging activity in cells

To evaluate the cytoprotective effects of GFNCs against H_2O_2 -induced damage in mice bone marrow cells, the FDC-P1 cells were firstly seeded into a 96-well plate with a density of ca. 1×10^4 cells per well in DMEM, then incubated with medium containing 100 $\mu\text{mol L}^{-1}$ of H_2O_2 using pure PBS as the control. After that, the medium containing H_2O_2 was replaced with the medium containing GFNCs solutions (with Gd^{3+} concentrations of 0–30 $\mu\text{mol L}^{-1}$). The cells were incubated with the GFNCs for 3 h in the dark at 37°C. Then the medium was removed again, and cells were cultivated in a culture medium for another 24 h in incubator at 37°C. Afterward, the cells viabilities were detected using CCK-8.

Biodistribution study *in vivo*

All animal operations were performed in accordance with the institutional animal use and care regulations of the Experimental Animal Center of Institute of Process Engineering, Chinese Academy of Sciences (CAS). The mice were sacrificed at predetermined times after injection of 1 mmol L⁻¹ GFNCs solution (150 μ L, 1 h and 24 h) through the tail vein. Then hind leg bones and bone marrows from animals of different groups were weighted and chemically digested. The digested samples were resuspended in 2% trace metal grade HNO₃ and filtered through 0.22- μ m pore size filter membrane (Millipore, USA). All samples were analyzed for the Gd³⁺ concentration by inductively coupled plasma mass spectrometry (ICP-MS, NexION 300X, Perkinelmer, USA).

Tumor models

Female ICR mice weighing 18–20 g were purchased from Peking University Health Science Center (Beijing, China). Four to five week old female ICR mice were injected in the right rear flank area with approximately 5×10^6 H22 mouse hepatoma cancer cells in 100 μ L of serum-free RPMI-1640 medium. The mice were anaesthetized by intraperitoneal injection of 100 μ L pentobarbital sodium (10%). Animals were maintained in our animal care facility and housed five per cage at room temperature (24 $\pm$ 2°C), food and water replaced every other day. It took 5–7 days for the tumor to grow up to approximately 5 mm after cells injection, and the treatments were conducted.

Electron microprobe analysis for Gd³⁺ in bone marrow

The analysis was carried out on polished thin sections using an electron microprobe (EMP, SHIMADZU EPMA-1720, Japan). All the bone marrow acquired from mice legs at different time points (0.5, 1, 4, and 24 h) post-injection of GFNCs was spread on conductive adhesive carbon film. Then the chemical compositions of different elements in the marrow were obtained using EMP in wavelength-dispersion mode. The operating conditions for the quantitative analyses were as follows: 30 kV accelerated voltage and 30 μ m beam diameter. All data were corrected using the relevant correction procedures. At the same time, the pure Gd@C₈₂ sample was tested using the same method above.

Bone marrow suppression and protection experiments

In the bone marrow suppression protection and tumor chemotherapy experiments, 18 tumor-bearing female ICR mice were randomly divided into three different experimental groups, including the control group, CTX-treated

group (60 mg kg⁻¹) by intraperitoneal injection (i.p.) and GFNCs (0.01 mmol L⁻¹ kg⁻¹) by intravenous injection (i.v.) + CTX-treated group (60 mg kg⁻¹) by i.p. injection with 6 mice in each group for five consecutive days. On day 4, 8, 10, 14, and 18, blood samples were quickly drawn from the orbit, which had no negative effect on the mice. Then 20 μ L blood samples from each tube were transferred to 3 mL centrifuge tubes for hematological analysis. Four important indicators (white blood cell count (WBC), hemoglobin level (HGB), lymphocyte count (LY) and monocyte percentage (MO%)) related to bone marrow inhibition were measured using an automated hematology analyzer (Celltac E MEK-7222K, NIHON KOHDEN, Japan).

The individual mice in the control group, CTX-treated and GFNCs + CTX treated groups were weighed every 2 days and their tumor sizes were measured. On day 18, blood samples were obtained from the mice prior to euthanasia *via* decapitation, and photographs of the mice as well as their tumors were taken. Blood samples were centrifuged at 3000 $\times$ g for 10 min to collect serum for blood biochemical tests. Indicators (alanine aminotransferase (ALT); aspartate aminotransferase (AST); alkaline phosphatase (ALP); lactate dehydrogenase (LDH); blood urea nitrogen (BUN); uric acid (UA)) for liver, heart and kidney functions were measured. The main organs were excised, weighed and fixed immediately in a 4% formaldehyde solution for histological study.

Environmental scanning electron microscopy (ESEM) and histopathological observation

Another 24 ICR mice were set into three groups, including groups of control, CTX (60 mg kg⁻¹) by i.p., and GFNCs (0.01 mmol L⁻¹ kg⁻¹) by i.v. + CTX (60 mg kg⁻¹) by i.p. injection with 8 mice in each group for consecutive five days. Then on day 4 and day 8, the mice were selected randomly for ESEM and H&E examination. To be specific, a whole portion of intact bone tissue containing the total marrow and niche, a small portion of liver, spleen, and 50 μ L of blood sample were macerated slowly in a physiological manner without damaging the inner cell mass and incubated in 2.5% glutaraldehyde overnight for fixation. To dry the tissues, they were repeatedly passed through 30%, 50%, 70%, 95%, and 100% gradients of alcohol, followed by critical point drying. Then, after coating the samples with gold (Au) IB-2 ions, the coated samples from the different groups underwent ESEM examination using an S-5330 Hitachi SEM, and the results were recorded.

Hematoxylin and eosin (H&E) staining was performed

following the standard protocols. Briefly, the organs of mice in the different groups were collected and immediately fixed in a natural 4% formalin buffered solution, embedded in paraffin, and cut into 5- μm thick sections. Then, the sections were stained with H&E staining for histological analysis, and the stained slices were examined using a Nikon Eclipse E400 microscope (Nikon France SA). The pathologist was blinded to the background and analysis of the slides.

Enzymatic assays

On day 4, 8, and 18, mice from each group were selected randomly to obtain the blood serum by extracting the eyeball blood. The level of antioxidant enzymes superoxide dismutase (SOD) was determined using the method of Kakkar *et al.* in which the inhibition of the formation of NADPH-phenazine methosulphate nitroblue tetrazolium formazone was measured spectrophotometrically at 560 nm. Lipid peroxidation (LPO), as expressed in malondialdehyde (MDA) levels, was determined using a method utilizing thiobarbituric acid reactive substances (TBARS). The concentration of MDA was expressed as nanomole per milligram of protein, using 1,1,3,3-tetraethoxy-propane (TEP) as the standard. Activity of catalase (CAT) was measured according to the method of Thomson *et al.* An assay to measure glutathione S-transferase (GST) activity was determined by the method of Habig *et al.* The level of Glutathione peroxidase (GP_x) was assayed based on the reaction between glutathione remaining after the action of GP_x and 5,5'-dithiobid-2-nitrobenzoic acid to form a complex that absorbs maximally at 412 nm. Results were expressed as relative activities between the experimental mice and the control group.

Statistical analysis

All of the data are presented as the mean $\pm$ standard deviation (SD). Statistical significance was evaluated with Student's *t* tests for comparisons between two groups using SPSS 19. A probability value of $P < 0.05$ was considered significant.

RESULTS

Characterization of GFNCs *in vitro* and cytoprotective study

The water-soluble GFNCs were prepared with a previously reported method [32]. Briefly, Gd@C_{82} powder was treated with H_2O_2 and NaOH solution to form Gd@C_{82} nanocrystals with multi-hydroxyl groups conjugating on the surface, as illustrated in Fig. 1a and labeled as GFNCs

hereafter. To characterize the size of water soluble GFNCs, 100 $\mu\text{mol L}^{-1}$ of GFNCs dissolved in 0.9% saline, AFM and DLS measurements were performed. The core size of the GFNCs was estimated ca. 30 nm based on the mean height of AFM images (Fig. 1b) and the average hydrodynamic diameter of the material was (DLS, Fig. 1c) ca. 120 nm.

The radical scavenging property of GFNCs *in vitro* was characterized by the ESR measurement. The $\bullet\text{OH}$ radicals were generated by the classic UV-induced H_2O_2 reaction (exposure to UV light for 8 min), and DMPO was selected as a spin trap agent. As shown in Fig. 1d, the typical four-line ESR signals of $\bullet\text{OH}$ radicals were largely decreased after 50 $\mu\text{mol L}^{-1}$ GFNCs solution being added. The strong $\bullet\text{OH}$ radicals scavenging property of GFNCs may be attributed to the large electron affinity of gadofullerenes, as well as the effect that the active hydroxyl radical would attack the hydroxyl protons in the GFNCs [41–43].

Several previous studies have already indicated that fullerene and gadofullerene derivatives could protect cells against the H_2O_2 damage through reducing the intracellular ROS level by using the human lung adenocarcinoma (A549) cells and the rat brain capillary endothelial cells (rBCECs) [35,44]. We want to study the myelosuppression protective effect of GFNCs in this study, so the mice bone marrow (FDC-P1) cells were adopted. As shown in Fig. 1e, it was observed that the GFNCs had negligible cytotoxicity to the FDC-P1 cells, and in the further cytoprotective experiment (Fig. 1f), the GFNCs exhibited a prominent protective effect against oxidative-induced cellular stress with the concentration from 2.5 up to 30 $\mu\text{mol L}^{-1}$. With 20 $\mu\text{mol L}^{-1}$ of the GFNCs, it even increased the activity of FDC-P1 cells up to 40.5%.

The bone marrow targeted distribution of GFNCs

In our previous paper, we studied the biodistribution of GFNCs in mice by the ^{131}I radio-labeled technique [32], and it was observed that the GFNCs were highly accumulated in the mice bone (Fig. S1). Here the atomic absorption spectrometry was used to further determine the detailed GFNCs biodistribution inside bone by ICP-MS. Firstly, the GFNCs concentrations in blood plasma were measured to determine the plasma half-life (Fig. 2a) to be ca. 4.3 h. Then the bone marrow was separated from the mice leg bones, and the concentrations of Gd^{3+} in the bone marrow and sclerotin were separately analyzed. In contrast to the decreasing trend of Gd^{3+} in blood plasma, the concentration of GFNCs was observed to increase in the bone in 24 h, and fascinatingly, the content of Gd^{3+} in the bone marrow was 10 times higher than that in the sclerotin (Fig. 2b).

Figure 1 Characteristics of GFNCs *in vitro*. (a) Schematic diagram of the as-synthesized GFNCs by hydroxylation and a photograph of the GFNCs aqueous solution. (b) AFM study of GFNCs to show the core size of the samples. (c) Average hydrodynamic diameter distributions of GFNCs in saline. (d) The ESR spectra of the hydroxyl radicals captured by DMPO after treatment with 50 $\mu\text{mol L}^{-1}$ of GFNCs (red line). The saline was used as a blank (black line). The hydroxyl radical was generated by the H_2O_2 exposure to UV light for 8 min. (e) The cell viability of mice bone marrow FDC-P1 cells incubated with the GFNCs (0.5–30 $\mu\text{mol L}^{-1}$) for 24 h. It shows no toxicity of GFNCs towards the FDC-P1 cells. (f) Cytoprotective effects of the GFNCs (0.5–30 $\mu\text{mol L}^{-1}$) against H_2O_2 -induced damage on the FDC-P1 cells ($n = 6$; *, $P < 0.05$).

To confirm the unusual distribution of GFNCs inside mice bone, the electron microprobe technique (Fig. 2c) was employed to further study the biodistribution of GFNCs. As shown in Fig. 2d, the content of Gd^{3+} in mice bone marrow gradually increased following the GFNCs being intravenously administered in mice. The concentrations of GFNCs in the mice bone marrow after 0.5, 1, 4, and 24 h administration were measured to be 0.58%

$\pm 0.02\%$, $1.33\% \pm 0.08\%$ (Fig. S2), $1.49\% \pm 0.07\%$, and $2.29\% \pm 0.11\%$ (Fig. 2d), respectively. These results are highly consistent with that of ICP-MS measurements in Fig. 2b.

GFNCs protect mice from CTX-induced bone marrow toxicity

CTX is widely applied in clinic for its high efficiency in tumor curing and its convenience in clinical using, but its

Figure 2 The biodistribution of GFNCs *in vivo*. (a) The GFNCs distribution in blood plasma of the test mice at different time points (5 min, 15 min, 30 min, 45 min, 1 h, 4 h, and 24 h) after a single i.v. injection of GFNCs (expressed as ng Gd³⁺ mg⁻¹ blood). (b) The GFNCs distribution in sclerotin and bone marrow of the test mice at 1 h and 24 h after administration with GFNCs ($n = 5$; *, $P < 0.05$). (c) Schematic illustration of the electron microprobe used to analyze the distribution of GFNCs in the mice bones. (d) The electron microprobe study about the time dependence of Gd³⁺ in mice bone marrow after GFNCs injection (0.5, 1, 4 and 24 h).

serious myelosuppression as well as other side effects such as immunosuppression, renal toxicity, neurotoxicity, emesis, and hearing loss, are also well known [21,45]. So here we select this medicine as the chemotherapeutic agent to test the protection effect of the GFNCs. Eighteen female ICR tumor-bearing mice implanted with H22 mouse hepatoma cancer were randomly separated into three groups consisting of saline control, CTX alone and GFNCs + CTX (Fig. 3a). In the chemotherapy protective experiments, the mice were administered with GFNCs (15 mg kg⁻¹) one day before the chemotherapy, from the second day the treatments with CTX (60 mg kg⁻¹) and GFNCs (15 mg kg⁻¹) started for consecutive five days. Beginning from the sixth day, all the three groups of mice were stopped to give any drugs, until the harvest day (18th day, Fig. 3a).

In order to quantitatively examine the chemotherapy protective effects, the routine blood tests were made in all the three groups on day 4, 8, 10, 14 and 18 after treatment. The routine blood tests were used to quantitatively examine the toxicity of the chemotherapy agents. Four representative hematological parameters including the

WBC, LY, HGB and MO% were used to evaluate the damage degree of bone marrow suppression, in which the WBC count would be the key clinical index for myelosuppression. The results of plasma tests of the three groups are shown in Fig. 3b, obviously, the administration of CTX led to a severe myelosuppression and caused a serious reduction of the total WBC, LY counts in the CTX alone group. As the red blood cells have longer life than that of the WBC, there was a slight reduction of the HGB level, and because of the myelodysplastic syndrome of myelosuppression mice, the MO% count in CTX treated-mice blood had an abnormal increase.

Comparing with the CTX alone group, the GFNCs + CTX group showed significant improvements on the WBC and LY counts in the therapeutic process. It was observed that the GFNCs + CTX group of mice had also a decrease in WBC, LY and HGB counts on the first several days during the CTX therapy, but the values recovered much faster than that in the CTX group. Especially, on day 8 of the therapy process, the counts of the WBC, LY and HGB in the GFNCs + CTX group were enhanced up to 166%,

Figure 3 The myelosuppression protective tests. (a) Schematic illustration of bone marrow protective process of the GFNCs. (b–e) Hematological parameters including WBC, HGB, LY and MO% in the control group, the CTX group and the GFNCs + CTX group during the chemoprotective tests. ($n = 6$; *, $P < 0.05$). (f) Activities of MDA, SOD, GST, CAT and GP_x in mice plasma on the day 18.

285% and 26% comparing with that in CTX group, respectively. As for the MO% value, it was observed that the GFNCs + CTX group was much closer to the normal level than the CTX group. Finally, after 18 days of the chemotherapy, it was found that nearly all the WBC, LY, HGB, and MO% values in the GFNCs + CTX group reached a similar level as the control group, while these parameters were still in abnormal status in the CTX group. Therefore, these results unambiguously revealed the effective protection of the myelosuppression by GFNCs.

On day 18, all the mice were harvested, thus we have enough blood plasma to measure the ROS-related enzymes *in vivo* with CTX and GFNCs treatments, five different enzymes, including MDA, SOD, GST, CAT, and GP_x, were measured. As shown in Fig. 3f, it was observed that the activities of the enzymes in the CTX group were a little higher than those of the control, while those in the GFNCs + CTX group had completely recovered to normal levels, further confirming the protective effect of the GFNCs to the CTX injury.

Figure 4 The antitumor activity of CTX and the GFNCs protection against CTX-induced toxicity in mice. (a) The growth inhibitory curves of tumors (left) and body weight changes (right) in the control group, CTX group and GFNCs + CTX group ($n = 6$; *, $P < 0.05$, compared with the control group). (b) Photos of tumors of 18 tested mice after chemotherapy in three different groups. (c) Typical biochemical parameters: ALT, AST, ALP, LDH, BUN and UA, which are relevant to liver (ALT, AST and ALP), heart (LDH) and kidney functions (BUN and UA) ($n = 6$; *, $P < 0.05$). (d) Selected H&E sections of major organs (heart, liver, spleen, lung and kidney) and tumor tissues from the tested mice in different groups.

GFNCs benefit the antitumor activity of CTX and reduce the toxicity induced by CTX in hepatoma bearing mice

Now we turned to the tumor therapeutic effects by CTX and GFNCs. Since both CTX and gadofullerenes were reported to own antineoplastic effects in previous studies [46,47], it is expected that the GFNCs + CTX group should have stronger anti-tumor effect. In the tumor therapeutic process, the tumor size and body weight of each mouse in all the three groups were measured every other days (Fig. 4a, b). The tumor growth inhibitory curves of GFNCs-treated group were shown in Fig. S3. As expected, the tumor growth curves clearly showed that the GFNCs indeed slightly enhanced the therapy effect of CTX to H22 tumor, although the enhancement was not statistically significant. The mice weights of CTX group were reduced from 21.5 ± 0.8 to 19.5 ± 1.4 g in the first 6 days, and those of

the GFNCs + CTX group were only slightly decreased from 21.6 ± 0.7 to 20.9 ± 1.9 g in the same period due to the protective effects of the GFNCs. After the CTX agents were stopped, there were quick increases in mice body weight in the CTX group and the GFNCs + CTX group. But until the end of experiments, the average body weight of the GFNCs + CTX group was much higher than that of CTX group. Besides myelosuppression, the CTX agent has also other side effects on the injury of normal organs, so six typical serum biochemistry indicators, i.e., the ALP, ALT, AST, BUN, LDH and UA, were measured to study the damages to normal organs and tissues. Among them, ALP, ALT and AST are mainly related to the hepatocellular functions, and the lower values of them in GFNCs + CTX group than that in CTX group indicate the liver protection of GFNCs to CTX injury; BUN and UA are

related to the renal functions of mice, and the lower levels of them in the GFNCs + CTX suggest that the GFNCs have also protection to kidney. Finally, the LDH level is related to the heart functions of mice, and the much lower LDH level in GFNCs + CTX group indicates that the GFNCs have positive functions to protect the mice hearts.

In fact, other than the above mentioned biochemical parameters in serum, the histological studies are more reliable in examining the CTX toxicity to mice organs. So the H&E staining was performed for the three groups of mice as shown in Fig. 4d. For the H&E staining of tumor slices from the different groups of mice, it was observed that the tumor cells in the control group showed activated proliferation with more blue dyeing nuclei. However, the nucleus of H22 cancer cells in the CTX group and GFNCs + CTX group became condensing and shrinking with dark dyeing, and even erythrocyte appeared in the intercellular substance, indicating the significant apoptosis and necrosis of cancer cells. Except for tumors, it is obvious that all the mice organs such as liver, heart, kidney, lung, and spleen in GFNCs + CTX group are similar as the control group and have no apparent histopathological abnormalities. By contrast, for the CTX group, it was observed that there were some obvious abnormal atrophy in the cardiac muscle tissues, evident necrotic tissue as well as red pulp damage in the spleen, and smaller alveolar septum and hydropic alveolar geometry in the lung sections. Thus, the GFNCs not only maintained the antitumor activity of CTX, but also protected against the CTX-induced toxicity.

The chemoprotective mechanism study by oxidative stress related enzyme activities and histological, morphological analyses

Until now we have demonstrated that the GFNCs could effectively protect the tumor-bearing mice against the CTX-induced injury during the chemotherapeutic process. However, it would be better if we can get more information on how the GFNCs protect the mice from the chemotherapeutic toxicity. Another 24 female ICR mice were selected to systematically study the side effects of CTX agent and the protection effects of GFNCs in two chemotherapeutic stages, i.e., the CTX using (day 4) stage and the third day after CTX being stopped (day 8). The mice group separations and the drug-given sequences are exactly the same with the previous tumor-bearing tests. Twelve female ICR mice were randomly separated into three groups (control, CTX, and GFNCs + CTX) and they were treated with CTX, GFNCs, or saline solution as shown in Fig. 3a, and then harvested at day 4. Another 12 ICR mice were similarly treated but harvested at day 8.

These tested mice were studied in detail *via* the biochemical, histological, and morphological techniques.

The biochemical examinations were first made to evaluate the oxidative stress state of the three groups of the tested mice treated at day 4. It is known that CTX would lead to higher oxidative stress in the bone marrow, blood, and liver in mice, which decreases the level of the antioxidant enzymes and increases the level of ROS *in vivo* [21]. The MDA level was served as a crucial biomarker of LPO to effectively reflect the cell damage by excessive free radical oxidation. As shown in Fig. 5a, the CTX indeed induced a higher level of MDA in the CTX group, and it was effectively reduced in the GFNCs + CTX group. As analyzed before, the efficient protection of GFNCs to CTX injury should be attributed to the strong radical scavenging function of the GFNCs.

Besides MDA, the other four well-known antioxidative enzymes, SOD, CAT, GP_x and GST, were also examined because they are usually involved in cellular defenses against toxic insults. These enzymes take functions by reducing H₂O₂ and superoxide radicals, protecting the cell from LPO, thus preserving the intact structure of the bone marrow cells and other tissues cells. Due to the large amount of free radicals induced by the CTX agent, it was observed that the GP_x, CAT, GST and SOD enzymes were largely consumed in scavenging the radicals (Fig. 5a). However, in the GFNCs + CTX group, the levels of enzymes were almost all up regulated after the administration of gadofullerenes because partial of the CTX-induced radicals had been scavenged by the GFNCs.

We further examined the pathological morphology of the three groups of mice harvested in day 4 by H&E staining (Fig. 5b). The normal mouse bone marrow contains lots of hematopoietic area and some fat tissues [48,49], but in the CTX group, the hematopoietic area was found to significantly decrease accompanying with the increasing of fat tissues due to the CTX induced myelotoxicity. On the contrast, the GFNCs + CTX group showed similar histological features as the control group, indicating that the hematopoietic cells were effectively protected by the GFNCs. Besides the bone marrow, it is well known that spleen is another important immune organ related to hematopoietic function and blood storage functions, as shown in Fig. 5c and Fig. S4, the histological study visualize obvious damages induced by CTX with broad necrotic tissue and red pulp damages, while the spleen is also highly protected in the GFNCs + CTX group.

Fig. 5d shows the ESEM studying of the bone marrow and the blood red cells in the tested mice. For the CTX group of mice on day 4, there appeared a large number of

Figure 5 Oxidative stress-related enzymes activities and H&E, ESEM analysis of mice tissues on the fourth day of CTX chemotherapy. (a) Activities of MDA, SOD, CAT, GP_x, and GST in control, CTX, and GFNCs + CTX groups of mice ($n = 4$; *, $P < 0.05$). (b) Light microscopy of H&E sections of mice femur bone, in which the bone (Bo) and bone marrow (BM) labeled different bone sections; (→ in red) labeled the normal bone marrow cells and hematopoietic cells, (→ in black) labeled the fatty infiltration of hematopoietic tissues. (c) Light microscopy of H&E sections of mice spleen, in which (→ in black) labeled the splenic red pulp and (→ in red) the splenic white pulp. (d) ESEM images of mice bone marrow, in which (→ in blue) labeled the adipocytes and (→ in red) labeled the necrotic cells by the CTX injury. (e) ESEM images of mice blood red cells. Many shriveled and abnormal red cells were observed in the CTX group of mice, and the blood red cells in the GFNCs + CTX group showed normal morphology as that in controls.

apoptotic, necrotic cells, and also abundant fat tissues in the bone marrow, where the apoptotic cells exhibited shrinkage, retraction of cell membrane, and the necrotic cells swelled with the formation of blisters. Moreover, the bone marrow not only lacked cellularity but also was full of hollow cavity-like areas. By contrast, the ESEM images of the bone marrow in the GFNCs + CTX group was similar to the normal bone marrow in the control group, showing more intact cell density and fewer apoptotic or necrotic cells. As for the blood red cells, although the counting of red cells by blood cell count instrument showed no significant reduction after CTX treatment in the CTX group of mice, it was shocked to observe the shriveled and abnormal morphology of many red cells (Fig. 5e). It was applauded that once again the blood red cells in the

GFNCs + CTX group showed normal morphology as that in the control group.

We further studied the three groups of tested mice harvested on day 8, i.e., 3 days after the CTX and GFNCs were stopped. As shown in Fig. 6a, the activities of all the five enzymes, i.e., MDA, GP_x, CAT, GST and SOD, in the GFNCs + CTX group have recovered from the CTX-induced injury because their levels are nearly the same with those in the control group. However, for the CTX group, these enzymes were still in a high level due to the serious oxidative stress induced by CTX agent. Here, it should be noted that contrary trends of the enzyme level for the CTX group to the control group were on the day 8 comparing with that on day 4. The reason for the abnormal behavior on the level of these enzymes is because the rapid increase

Figure 6 Oxidative stress-related enzyme activities and H&E, ESEM analyses of mice tissues on the eighth day of the CTX therapy. (a) Activities of MDA, SOD, CAT, GP_x and GST in control, CTX, and GFNCs + CTX groups. ($n = 4$; *, $P < 0.05$). (b) Light microscopy of H&E sections of mice femur bone on the eighth day, in which the bone (Bo) and bone marrow (BM) labeled different bone sections; (→ in red) labeled the normal bone marrow cells and hematopoietic cells, (→ in black) labeled the fatty infiltration of hematopoietic tissues. (c) Light microscopy of H&E sections of mice spleen, in which (→ in black) labeled the splenic red pulp and (→ in red) the splenic white pulp. (d) ESEM images of mice bone marrow, in which (→ in blue) labeled the adipocytes and (→ in red) labeled the necrotic cells by the CTX injury. (e) ESEM images of mice blood red cells, in which the shrunken and abnormal red cells were mainly observed in the CTX group of mice.

of free radicals induced by the CTX agent largely consumed the antioxidant enzymes on the first several days, inducing the decreased levels of these enzymes on day 4, then it activated the biosynthesis of antioxidative enzymes by the compensative response on the following days in the CTX therapeutic process, leading to a rapid increase of the enzyme levels. Until day 5 the CTX agent was stopped, the CTX-induced radicals in the mice rapidly decreased to consume fewer enzymes, but it often takes several days to reach a dynamic balance between the amount of free radicals and the enzyme levels, so these oxidant-relevant enzymes were observed still in high levels on day 8. On contrast, in the CTX + GFNCs group, the gadofullerenes scavenged lots of the excessive CTX-induced free radicals, so the abnormal increase of the enzyme levels is not as

serious as that in CTX group, and it will recover quickly after stopping to use the CTX.

Fig. 6b–d showed the comparing H&E and ESEM studies of the bone marrow, spleen, and the red cells in the control, CTX and CTX+GFNCs groups. Comparing with 4 days ago, it can be observed that the bone marrow and spleen in CTX group were partially recovered, though large areas of fat tissues were still observed. For the CTX + GFNCs group, it is applauded that the CTX-induced injury was nearly disappeared, with hematopoietic cells and megakaryocytes in bone marrow tissues were significantly increased. And similar behavior was observed for the blood red cells as shown in Fig. 6e, where lots of abnormal cells still existed in the CTX group, but the blood cells in the CTX + GFNCs group showed little differences with

Figure 7 The schematic representation of the GFNCs protective mechanism related to bone marrow enrichment and free radical scavenging.

that in the control group.

DISCUSSION

Based on the above comparing experiments, the hematological studies on the WBC, HGB, LY and MO% parameters revealed a serious CTX injury on the tested mice and the administration of GFNCs could largely retrieve the myelosuppression, and H&E and ESEM studies on the mice tissues as well as on the morphology of blood cells revealed that the CTX seriously damage the spleen, lung, bone marrow and blood red cells. However, with the applying of GFNCs the injury were efficiently alleviated, and the biochemical studies on the oxidative stress-related enzymes after CTX therapy showed that GFNCs agent could scavenge these excess radicals and thus decrease the CTX side effects largely. Besides, the administration of the gadofullerenes not only protects against chemotherapeutic agent injury, but also maintains the anti-tumor properties of the drug.

The possible mechanism of GFNCs protecting against the chemotherapy injury was described in Fig. 7. First, when the GFNCs were intravenously injected, they were supposed to penetrate the sinusoid capillary to enter the bone marrow tissues. There were some nanosized inter-

spaces in the blood sinus structure (the amplifying structure in the schema). As the GFNCs were rigid nano-structure, it can more easily go through the blood sinus, thus achieving bone marrow accumulation in mice. Next, the CTX acted on the myeloid cells to generate lots of free radicals, resulting in a random damage to proteins, lipids, DNA and the hematopoietic tissues. However, in the GFNCs + CTX group, partial of the excess free radicals were scavenged, leading to less injury to the bone marrow cells. Similar protection effects of GFNCs to CTX injury on spleen, blood red cells, and other organs could also be explained in a same mechanism.

CONCLUSIONS

We demonstrated that the GFNCs could significantly reduce the chemotherapy injury caused by the clinical chemotherapeutic agent CTX, and maintained the high antitumor effect of this chemotherapeutic drug in the meantime. The GFNCs could be selectively accumulated into the bone marrow that would largely decrease the CTX-induced myelosuppression. The extra protective effects of the GFNCs to spleen and other organs were also observed. The chemoprotective activity of the GFNCs may be attributed to its antioxidant capacity to enhance he-

matopoietic function and to modulate organism immunity, although the exact underlying mechanism is still in progress. In summary, we presented a unique new way to overcome the serious toxicity from conventional chemotherapy agent, which would allow the safe use of increased anti-tumor drugs for the benefit of future cancer patients in clinic.

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- 1 André N, Carré M, Pasquier E. Metronomics: towards personalized chemotherapy? *Nat Rev Clin Oncol*, 2014, 11: 413–431
- 2 Kurtova AV, Xiao J, Mo Q, *et al.* Blocking PGE2-induced tumour repopulation abrogates bladder cancer chemoresistance. *Nature*, 2014, 517: 209–213
- 3 Fu D, Calvo JA, Samson LD. Balancing repair and tolerance of DNA damage caused by alkylating agents. *Nat Rev Cancer*, 2012, 52
- 4 Helleday T, Petermann E, Lundin C, *et al.* DNA repair pathways as targets for cancer therapy. *Nat Rev Cancer*, 2008, 8: 193–204
- 5 Costa L, Major PP. Effect of bisphosphonates on pain and quality of life in patients with bone metastases. *Nat Clin Prac Oncol*, 2009, 6: 163–174
- 6 Lachmann N, Czarnecki K, Brenning S, *et al.* Deoxycytidine-kinase knockdown as a novel myeloprotective strategy in the context of fludarabine, cytarabine or cladribine therapy. *Leukemia*, 2015, 29: 2266–2269
- 7 Das UB, Mallick M, Debnath JM, Ghosh D. Protective effect of ascorbic acid on cyclophosphamide-induced testicular gametogenic and androgenic disorders in male rats. *Asian J androl*, 2002, 4: 201–207
- 8 Lucas D, Scheiermann C, Chow A, *et al.* Chemotherapy-induced bone marrow nerve injury impairs hematopoietic regeneration. *Nat Med*, 2013, 19: 695–703
- 9 Levesque JP, Winkler IG. It takes nerves to recover from chemotherapy. *Nat Med*, 2013, 19: 669–671
- 10 Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat Rev Drug Discov*, 2009, 8: 579–591
- 11 Dvash E, Har-Tal M, Barak S, *et al.* Leukotriene C4 is the major trigger of stress-induced oxidative DNA damage. *Nat Commun*, 2015, 6: 10112
- 12 Macleod KF. The role of the RB tumour suppressor pathway in oxidative stress responses in the haematopoietic system. *Nat Rev Cancer*, 2008, 8: 769–781
- 13 Lin W, Yuan N, Wang Z, *et al.* Autophagy confers DNA damage repair pathways to protect the hematopoietic system from nuclear radiation injury. *Sci Rep*, 2015, 5: 12362–12373
- 14 Kerbel RS, Kamen BA. The anti-angiogenic basis of metronomic chemotherapy. *Nat Rev Cancer*, 2004, 4: 423–436
- 15 Manda K, Bhatia AL. Prophylactic action of melatonin against cyclophosphamide-induced oxidative stress in mice. *Cell Biol Toxicol*, 2003, 19: 367–372
- 16 Patra K, Bose S, Sarkar S, *et al.* Amelioration of cyclophosphamide induced myelosuppression and oxidative stress by cinnamic acid. *Chemico-Biol Interactions*, 2012, 195: 231–239
- 17 Xue Y, Lim S, Yang Y, *et al.* PDGF-BB modulates hematopoiesis and tumor angiogenesis by inducing erythropoietin production in stromal cells. *Nat Med*, 2011, 18: 100–110
- 18 Passequé E, Ernst P. IFN- α wakes up sleeping hematopoietic stem cells. *Nat Med*, 2009, 15: 612–613
- 19 Finkel T. Oxidant signals and oxidative stress. *Curr Opin Cell Biol*, 2003, 15: 247–254
- 20 Winterbourn C. Oxidative denaturation in congenital hemolytic anemias: the unstable hemoglobins. *Semin Hematol*, 1990, 27: 41–50
- 21 Lee J, Lim KT. Protection against cyclophosphamide-induced myelosuppression by ZPDC glycoprotein (24 kDa). *Immunol Invest*, 2013, 42: 61–80
- 22 Kawano M, Mabuchi S, Matsumoto Y, *et al.* The significance of G-CSF expression and myeloid-derived suppressor cells in the chemoresistance of uterine cervical cancer. *Sci Rep*, 2016, 5: 18217
- 23 Bakanay M, Demirel T. Novel agents and approaches for stem cell mobilization in normal donors and patients. *Bone Marrow Transplant*, 2012, 47: 1154–1163
- 24 Afifi S, Adel NG, Devlin S, *et al.* Upfront plerixafor plus G-CSF versus cyclophosphamide plus G-CSF for stem cell mobilization in multiple myeloma: efficacy and cost analysis study. *Bone Marrow Transplant*, 2016, 51: 546–552
- 25 Barreto JA, O'Malley W, Kubeil M, *et al.* Nanomaterials: applications in cancer imaging and therapy. *Adv Mater*, 2011, 23: H18–H40
- 26 Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer*, 2005, 5: 161–171
- 27 Wicki A, Witzigmann D, Balasubramanian V, *et al.* Nanomedicine in cancer therapy: challenges, opportunities, and clinical applications. *J Control Release*, 2015, 200: 138–157
- 28 Sayes CM, Marchione AA, Reed KL, *et al.* Comparative pulmonary toxicity assessments of C₆₀ water suspensions in rats: few differences in fullerene toxicity *in vivo* in contrast to *in vitro* profiles. *Nano Lett*, 2007, 7: 2399–2406
- 29 Aschberger K, Johnston HJ, Stone V, *et al.* Review of fullerene toxicity and exposure—appraisal of a human health risk assessment, based on open literature. *Regul Toxicol Pharmacol*, 2010, 58: 455–473
- 30 Yan L, Zhao F, Li S, *et al.* Low-toxic and safe nanomaterials by surface-chemical design, carbon nanotubes, fullerenes, metallofullerenes, and graphenes. *Nanoscale*, 2011, 3: 362–382
- 31 Wang J, Chen C, Li B, *et al.* Antioxidative function and biodistribution of [Gd@C₈₂(OH)₂₂]_n nanoparticles in tumor-bearing mice. *Biochem Pharmacol*, 2006, 71: 872–881
- 32 Zhen M, Shu C, Li J, *et al.* A highly efficient and tumor vascular-targeting therapeutic technique with size-expandable gadofullerene nanocrystals. *Sci China Mater*, 2015, 58: 799–810
- 33 Cagle DW, Kennel SJ, Mirzadeh S, *et al.* *In vivo* studies of fullerene-based materials using endohedral metallofullerene radio-tracers. *Proc Natl Acad Sci USA*, 1999, 96: 5182–5187
- 34 Gharbi N, Pressac M, Hadchouel M, *et al.* Fullerene is a powerful antioxidant *in vivo* with no acute or subacute toxicity. *Nano Lett*, 2005, 5: 2578–2585
- 35 Yin JJ, Lao F, Fu PP, *et al.* The scavenging of reactive oxygen species and the potential for cell protection by functionalized fullerene materials. *Biomaterials*, 2009, 30: 611–621
- 36 Duarte JH. Experimental arthritis: fullerene nanoparticles ameliorate disease in arthritis mouse model. *Nat Rev Rheumatol*, 2015, 11: 319–319

- 37 Xu JY, Su YY, Cheng JS, *et al.* Protective effects of fullereneol on carbon tetrachloride-induced acute hepatotoxicity and nephrotoxicity in rats. *Carbon*, 2010, 48: 1388–1396
- 38 Milic VD, Stankov K, Injac R, *et al.* Activity of antioxidative enzymes in erythrocytes after a single dose administration of doxorubicin in rats pretreated with fullereneol C₆₀(OH)₂₄. *Toxicol Mech Methods*, 2009, 19: 24–28
- 39 Baati T, Bourasset F, Gharbi N, *et al.* The prolongation of the lifespan of rats by repeated oral administration of fullerene. *Biomaterials*, 2012, 33: 4936–4946
- 40 Ji ZQ, Sun H, Wang H, *et al.* Biodistribution and tumor uptake of C₆₀(OH)_x in mice. *J Nanopart Res*, 2006, 8: 53–63
- 41 Zheng J, Zhen MM, Ge JC, *et al.* Multifunctional gadofulleride nanoprobe for magnetic resonance imaging/fluorescent dual modality molecular imaging and free radical scavenging. *Carbon*, 2013, 65: 175–180
- 42 Markovic Z, Trajkovic V. Biomedical potential of the reactive oxygen species generation and quenching by fullerenes (C₆₀). *Biomaterials*, 2008, 29: 3561–3573
- 43 Andrievsky GV, Bruskov VI, Tykhomyrov AA, *et al.* Peculiarities of the antioxidant and radioprotective effects of hydrated C₆₀ fullerene nanostructures *in vitro* and *in vivo*. *Free Radical Biol Med*, 2009, 47: 786–793
- 44 Karakoti A, Singh S, Dowding JM, *et al.* Redox-active radical scavenging nanomaterials. *Chem Soc Rev*, 2010, 39: 4422–4432
- 45 Lee HJ, Selesniemi K, Niikura Y, *et al.* Bone marrow transplantation generates immature oocytes and rescues long-term fertility in a preclinical mouse model of chemotherapy-induced premature ovarian failure. *J Clin Oncol*, 2007, 25: 3198–3204
- 46 Wang H, Agarwal P, Zhao S, *et al.* Combined cancer therapy with hyaluronan-decorated fullerene-silica multifunctional nanoparticles to target cancer stem-like cells. *Biomaterials*, 2016, 97: 62–73
- 47 Sun M, Kiourti A, Wang H, *et al.* Enhanced microwave hyperthermia of cancer cells with fullerene. *Mol Pharm*, 2016, 13: 2184–2192
- 48 Naveiras O, Nardi V, Wenzel PL, *et al.* Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. *Nature*, 2009, 460: 259–263
- 49 Cao S, Durrani FA, Tóth K, *et al.* Se-methylselenocysteine offers selective protection against toxicity and potentiates the antitumour activity of anticancer drugs in preclinical animal models. *Br J Cancer*, 2014, 110: 1733–1743

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Author contributions Zhang Y, Zhen M and Wang C performed the experiments, collected and analyzed the data, and wrote the manuscript; Shu C provided critical comments on the design of the study and the writing of the manuscript; Li J and Yu T performed the preparation of gadofullerenes nanocrystals (GFNCs); Jia W, Li X, Deng R, and Zhou Y provided essential technical assistance with experiments; Wang C conceived of and designed the study, supervised the research and wrote the manuscript. All authors discussed the results and approved the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

Supplementary information Experimental details and supporting data are available in the online version of the paper.

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一种新型靶向骨髓的金属富勒烯用于防护化疗造成的氧化损伤

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摘要 本文报道了一种利用金属富勒烯纳米材料实现肿瘤化疗骨髓保护的新技术。肿瘤化疗往往会带来严重的骨髓抑制等毒副作用, 给患者造成极大的创伤。利用金属富勒烯纳米材料高效清除自由基、高选择性且快速富集在小鼠骨髓中的特性, 开发了基于金属富勒烯的新型化疗辅助药物。研究表明, 金属富勒烯实现了多方面的化疗保护效果, 对白细胞计数最高可提升166%, 对淋巴细胞最高可提升285%, 对于骨髓以及脾脏多器官均有显著保护效果, 且未影响化疗药物的肿瘤治疗效果。该材料是一种快速、高效、毒副作用小的新型肿瘤化疗辅助药物, 具有巨大的发展潜力。