Efficient removal of uranium from mice by a novel compound of fullerence multi-macrocyclic polyamine derivatives postprint

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Abstract

Uranium removal efficacy of fullerence multi-macrocyclic polyamine derivatives (C60-MMP), a novel chelating agent, was evaluated in mice. C60-MMP was administrated intravenously into mice at 30 min after the uranium contamination. The molar ratio of chelating ligand/uranium was about 1:1. The results indicate that C60-MMP can effectively prevent accumulation of uranium in liver at 8 h after C60-MMP injection. At 48 h after the last injection, uranium deposition in liver of C60-MMP treated mice is approximately 65% less than that of the control group. C60-MMP reacted positively in promoting the removal of uranium from kidney, and the urinary uranium excretion increased significantly, compared with the control and DTPA-treated mice. However, repeated administration of C60-MMP, and combined injection of DTPA and C60-MMP, did not show desirable effects on uranium removal from mice. It implies that more investigations are needed for the treatment protocols and clinical applications of C60-MMP.

Full Text

Preamble

Efficient Removal of Uranium from Mice by a Novel Compound of Fullerene Multi-Macrocyclic Polyamine Derivatives

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Abstract: The uranium removal efficacy of fullerene multi-macrocyclic polyamine derivatives (C60-MMP), a novel chelating agent, was evaluated in mice. C60-MMP was administered intravenously 30 minutes after uranium contamination at a chelating ligand-to-uranium molar ratio of approximately 1:1. The results indicate that C60-MMP can effectively prevent uranium accumulation in the liver at 8 hours post-injection. At 48 hours after the final injection, uranium deposition in the livers of C60-MMP-treated mice was approximately 65% lower than that in the control group. C60-MMP demonstrated positive effects in promoting uranium removal from the kidneys, with urinary uranium excretion increasing significantly compared to both control and DTPA-treated mice. However, repeated administration of C60-MMP and combined injection of DTPA and C60-MMP did not produce desirable effects on uranium removal, suggesting that further investigation is needed regarding treatment protocols and clinical applications of C60-MMP.

Keywords: Uranium, Internal contamination, Removal, Fullerene multi-macrocyclic polyamine derivatives (C60-MMP)
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Introduction

As a long-lived, naturally occurring radioelement, uranium is widely used as nuclear fuel in fission reactors and weapons. It is also a hazardous heavy metal with serious biological/chemical toxicity and high radioactivity. Accidental intake of uranium by workers or the general public may occur frequently during extraction and purification, industrial manufacture and use of uranium compounds, and fabrication of nuclear fuels. Such acute or chronic exposures can lead to internal contamination that induces both heavy-metal chemical and radiological toxicities [**?**, **?**]. Numerous animal and human studies have shown that for soluble uranium compounds in vivo, the major harmful effect is chemical toxicity rather than radiotoxicity [**?**].

Although final conclusions cannot yet be drawn regarding cancer risks from uranium internal exposure, the potential health hazards of uranium were recognized in the early days of its application [**?**]. An increasing number of investigations into the pharmacology and toxicology of uranium compounds demonstrate that uranium is harmful to bone, kidney, liver, and the central nervous system, and it perturbs the antioxidant defense system and other bodily functions in humans

[?]. The appropriate measures for uranium contamination depend largely on the intake pathway (inhalation, ingestion, or wound), the level of exposure, and treatment delay. For inhalation contamination, blood chelation and lung washing are recommended. For ingestion, proposed measures include gastric lavage, precipitation, purgatives, and chelation of the absorbed fraction in blood. For uranium entering via wounds, blood chelation, surgical excision, and washing are advised. Therefore, chelation therapy is the most universal and available treatment to alleviate uranium toxicity in any case [?, ?].

Ideal antidotes for uranium intoxication should form excretable uranium complexes of high stability in tissues and body fluids, since biological ligands compete for uranium complexation under physiological conditions. For in vivo application, such antidotes should have desired solubility and low toxicity at effective dosages [?, ?]. Additionally, it is highly desirable that the chelating agent be orally available for long-term therapy. An effective chelating agent can reduce uranium retention on bone surfaces and in soft tissues by re-circulated uranium [?]. Considerable decorporation agents have been designed and evaluated for actinide chelation therapy [?]. The Radiation Laboratory at the University of California, Berkeley has been designing improved actinide-sequestering agents for chelation therapy since the early 1950s [?]. A library of catecholamide (CAM), terephthalamide (TAM), and hydroxypyridinone (HOPO) ligands has been investigated for decontamination of Pu(IV), Th(IV), Am(III), uranyl ion, and Np(IV), among others [?, ?]. In particular, two selected chelating agents, 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO), are considered effective for decorporation of Pu(IV), Am(III), U(VI)O_2, Np(V)O_2, and are much more effective than diethylenetriaminepentaacetate (DTPA), the clinically approved chelator [?, ?, ?, ?]. Stradling and co-workers conducted investigations on actinide decorporation, particularly for chelation of Pu(IV), Am(III), U(VI), and Th(IV) with DTPA and 3,4,3-LIHOPO [?]. Domingo et al. studied the removal efficacy of 4,5-dihydroxy-1,3-benzenedisulfonic acid (Tiron) in protecting against uranium toxicity in vivo, demonstrating that Tiron was unparalleled in reducing uranium levels in kidney and bone. Unfortunately, this chelator failed to reduce skeletal uranium deposition when injection was delayed [?, ?, ?, ?]. Fukuda et al. investigated the chelating efficacy of bicarbonate combined respectively with ethane-1-hydroxy-1,1-bisphosphonate (EHBP), deferiprone (L1), and other chelators in removing depleted uranium (DU) [?]. However, these chelating agents can serve only as investigative drugs that are incompatible with reducing bone deposition, soft tissue burden, and uranium excretion.

It is reported that fullerene and its derivatives tend to distribute in kidney, liver, and bone [?]. In this work, seeking an effective uranium-sequestering agent, we synthesized several new compounds by introducing functional groups (such as cyclen and pyrocatechol) into fullerenes. Among them, fullerene multimacrocyclic polyamine derivative (C60-MMP, n = 2, Wm = 1887.53; n = 3, Wm = 2470.98, Fig. 1 [Figure 1: see original paper]) may be a possible biomimetic uranium chelator based on preliminary tests of toxicity, tissue biodistribution, and blood clearance rate in vivo.

Fig. 1. Chemical structure of fullerene multi-macrocyclic polyamine derivatives (C60-MMP).

In this work, C60-MMP was further evaluated as a potential biomimetic uranium chelator. The uranium biodistribution and uranyl removal efficacy of C60-MMP were investigated in uranium-contaminated mice, and the chelating ligand potency was evaluated by comparing uranium retention and distribution in C60-MMP-treated mouse groups with uranyl control and DTPA-treated groups. Given that therapeutic effects may be affected by the exposure pathway and chelation treatment protocol, comparative experiments were carried out between chronic low-dose and acute high-dose uranium poisoning mice, and between single and repeated injections. Additionally, the decorporation efficacy of using a combined drug (C60-MMP and DTPA) in chelation therapy was evaluated by comparing it with the efficacy of injecting C60-MMP or DTPA alone.

Materials and Methods

A. Chemicals and Animals

DTPA, obtained from Shanghai Chemical Reagent Supply Depot (Shanghai, China), was dissolved in 0.9% saline solution, with pH adjusted to 7.4–8.4 using NaOH solution. Uranyl nitrate (Luxembourg, Germany) was prepared by dissolving uranyl nitrate hexahydrate in distilled water, and the solution was adjusted to pH 7 just before administration to mice. Since the uranyl ion (UO_2^{2+}) is the most stable form in which uranium exists in biological systems [**?**, **?**, **?**], uranium was administered as uranyl nitrate. Solution concentrations were adjusted to permit a dosing volume of 0.1 mL for injection.

C60-MMP was synthesized in the Department of Chemistry, Sichuan University (Chengdu, China). It was dissolved in 0.9% saline solution, with pH adjusted close to body fluid.

The experimental animals were adult Kunming mice weighing 18–22 g (Huaxi Medical College, Sichuan University, Chengdu, China). They were used in accordance with guidelines on the care and use of laboratory animals. The 144 mice were housed in metabolic cages in groups of eight after uranyl contamination. They were maintained on an ad libitum diet and water, at relative humidity of 30%–70% and ambient temperature of 22–25 °C.

B. Determination of Uranyl-Removal Efficacy

Mice were divided into 18 groups: six control groups (uranium-contaminated only), six positive control groups (DTPA-treated), and six experimental groups (C60-MMP-treated). Each mouse received a dose of uranyl nitrate (0.1 mg) via lateral tail vein injection. Positive control and experimental groups were injected intravenously (i.v.) with a single dose of DTPA or C60-MMP, respectively, 30 minutes after uranyl nitrate contamination. The ligands were injected at

a ligand-to-uranium molar ratio of approximately 1:1 (about one-sixth of the intravenous LD_{50} value of DTPA and half of the intravenous LD_{50} value of C60-MMP). Mice were humanely euthanized at 4, 8, 16, 24, and 48 hours post-contamination. Indicative tissues and organs (blood, liver, kidney, and skeleton) were obtained. However, the 0.5-hour groups were used for blood collection only. Urine and feces were collected from groups sacrificed at 24 and 48 hours after the final injections to measure fecal elimination of uranium.

C. Effect of Repeated Administration

The efficacy of repeated low-dose chelating ligand administration on uranium degradation was measured by comparing uranium biodistribution and fecal excretion in mice receiving only a single high-dose chelator injection. Two groups of mice received a single dose of DTPA (0.33 mol per mouse) or C60-MMP (0.33 mol per mouse) at 30 minutes post-contamination with uranyl nitrate. Another two groups received three injections of DTPA or C60-MMP (0.11 mol per animal per dose) at 0.5, 4, and 8 hours after intravenous contamination. Mice not receiving chelation therapy were kept in metabolic cages for urine and feces collection. Mice were sacrificed 24 hours after intravenous $UO_2{}^{2+}$ injection, and blood, liver, kidney, and bone were removed.

D. Efficacy of Chelation Therapy with Combined Drugs

Mice were divided into three groups. Group 1 received 0.17 mol DTPA and 0.17 mol C60-MMP sequentially at 30 minutes after uranium contamination. Groups 2 and 3 were treated with 0.33 mol DTPA and C60-MMP, respectively, after uranium injection. Urine and feces were collected, and mice were sacrificed 24 hours after the final injection. Blood, liver, kidney, and bone were immediately removed.

E. Efficacy of Chelation Therapy in Degrading Chronic Uranium Toxicity

Twenty-four mice were contaminated with uranyl nitrate once weekly for 4 weeks, with each mouse receiving a tail intravenous injection of 0.08 mol. Mice were then divided into three groups and housed separately in metabolic cages. The first group received 0.1 mL of 0.9% saline, while the other two groups received chelation therapy with DTPA and C60-MMP at 0.33 mol per mouse, respectively. To ensure results reflected the potential efficacy of chelators at removing deposited U(VI) from target tissues like liver, kidney, and bone, chelating ligands were administered 48 hours after the final uranyl nitrate injection. Mice were sacrificed 24 hours after chelation therapy, and blood, liver, kidney, and bone were collected.

F. Tissue Processing and Measurement

Wet samples were weighed just prior to pretreatment. Blood, livers, kidneys, and bones were processed as individual samples, while separated urine and feces were pooled for each group. Tissues and feces were first dried at 200 °C and dry-ashed at 850 °C. The ashed samples were then treated using wet digestion (mixed with concentrated nitric acid and hydrogen peroxide and heated at 200 °C). Urine samples were processed directly with concentrated nitric acid and hydrogen peroxide. Uranium concentrations in the prepared samples were analyzed using WGJ-III laser-induced fluorescence (Hangzhou, China).

G. Data Management and Analysis

Final experimental data are expressed as uranium content per gram of wet tissue weight (g U/g b.wt.), presented as arithmetic means ± standard deviation. Each group was measured as a complete metabolic balance study. When comparing values between groups, the term "significant" is used in the statistical sense, indicating $P < 0.05$ by one-way analysis of variance (ANOVA) followed by appropriate post hoc analysis. Treatment groups were compared with controls using Student' s t-test [**?**, **?**].

Results and Discussion

A. Effectiveness of C60-MMP in Uranium Decorporation

Investigations of C60-MMP uranium decorporation efficacy were performed in experimentally acute uranium-poisoned mice. Chelating ligand potency was evaluated by comparing uranium retention in tissues and excretion in excreta of chelator-treated mice with corresponding control groups contaminated with uranyl nitrate only and with groups similarly treated with DTPA.

Following intravenous injection, uranium should be absorbed into the blood within a short time (10–30 minutes) before distribution to target organs. If uranium is injected via tail vein, about two-thirds of UO_2^{2+} is eliminated from plasma. Apart from renal excretion, approximately 20% of total uranium deposits in the skeleton and 12% in the kidneys, with the liver being another major damaged organ [**?**, **?**]. Considering these facts and preliminary biodistribution results, blood, liver, kidney, and skeleton were selected to assess the decorporation efficacy of the new chelating ligand.

Biodistribution of uranium in blood and tissues was determined at different time intervals after the final injection. The data were used to analyze uranium metabolism in vivo and changes in uranium concentration in organs and tissues after chelation therapy.

Uranium concentration changes in blood (Fig. 2 Figure 2: see original paper) showed that UO_2^{2+} remained in blood for a relatively longer time (8–16 hours) in C60-MMP-treated mice. Uranium concentration increased during

the first eight hours, whereas uranyl ion concentrations in blood of untreated control mice and DTPA-treated mice decreased to background levels within a short transition period. The increased uranium concentrations in blood of the C60-MMP-treated group during the first eight hours may contribute to reduced uranium concentration in the kidney. Apart from urinary excretion, uranyl ions partially re-enter blood during recirculation. This also indicates that uranium in blood is distributed slowly into target organs, which is conducive to chelator antidotal efficacy. For all three groups, blood uranium concentrations at 48 hours were higher than at 24 hours, with significant increases in both DTPA-treated (146%) and C60-MMP-treated (86%) groups. This suggests that, compared with the control group, a larger portion of uranyl ions deposited in target organs of treated mice may recirculate in blood.

The liver is another indicative sample of uranium toxicity, and uranium removal from the liver is an important criterion for chelating ligand decorporation efficacy. Liver uranium metabolism over 48 hours is shown in Fig. 2 Figure 2: see original paper. The efficacy of C60-MMP in decorporating uranium from livers is quite obvious from 8 hours post-injection of the chelating ligand. At 48 hours, the average uranium content in livers of C60-MMP-treated mice was 65% lower than the control group and nearly 70% lower than the DTPA-treated group. Together with the results of uranium concentration variations in blood, it is safe to conclude that the longer residence time of uranium in blood helps prevent metal ion deposition in the liver, thereby reducing the difficulty of sequestering uranyl ions. Regarding DTPA' s effect on degrading uranium poisoning in contaminated mice, the results are rather disappointing. Compared with U(VI) control groups, DTPA is not only ineffective in liver protection but also increases uranium retention, consistent with experimental results from Durbin and Ortega et al. [**?**, **?**, **?**].

Figure 2 Figure 2: see original paper shows uranium concentrations in kidneys at various times after C60-MMP injection. Uranium metabolism in kidneys of C60-MMP-treated mice presents different tendencies. Uranyl ion concentrations in kidney remained relatively high for 16 hours after injection. However, high uranium concentrations in kidneys of U(VI) and DTPA-treated control groups were maintained only for a relatively short period, ranging from 4 to 8 hours. Furthermore, significant reductions in uranium deposition in kidneys were observed in both chelation-treated mouse groups compared with the U(VI) control group. This indicates that most uranyl ions formed excretable compounds with chelators and were expelled from the body in urine, since renal excretion is the major route of uranium elimination [**?**, **?**].

Nevertheless, both DTPA and C60-MMP failed to remove uranium already deposited in bone (Fig. 2 Figure 2: see original paper). At 48 hours after chelation therapy, the increased U(VI) in skeleton of C60-MMP-treated mice may contribute to understanding uranium recirculation in mice. It was reported that other chelating ligands, such as gallic acid, EDTA, and 3-LI(Me-3,2-HOPO), had similar results [**?**, **?**].

A potential sequestering agent reduces uranium retention in organs and increases excreta elimination. Urinary excretion data (Table 1) demonstrate the therapeutic efficacy of chelators in promoting uranium excretion, with C60-MMP being much more effective than DTPA in increasing urinary excretion. As shown in Table 1, urine rather than feces is the main route of uranium excretion from the body, as previously reported [**?**, **?**].

The efficacy of C60-MMP in uranium chelation may be due to the fact that C60-MMP has multiple uranium-binding units, and this uranium-binding unit, polyamine, can form rather stable complexes with uranyl ions [**?**, **?**].

B. Effects of Repeated Chelator Administration and Combined Drugs on Chelation Therapy of Uranium Toxicity

Good efficacy with repeated low-dose administration is a desirable property of a chelating ligand, as high chelator doses are toxic to humans. The effectiveness of repeated treatment in poisoned mice with sequestering agents was evaluated, with results shown in Fig. 3 Figure 3: see original paper. When administered three times at the same total dosage, DTPA resulted in significantly lower uranium depositions in liver, kidney, and skeleton compared with a single injection. Repeated low-dose injection of C60-MMP showed slight efficacy in reducing renal uranium but markedly increased uranium retention in the skeleton. Additionally, both DTPA and C60-MMP were almost ineffective at promoting urinary uranium excretion (Table 2). Together with the results of repeated DTPA and C60-MMP administration, we can draw the preliminary conclusion that DTPA is effective at low dosage, while C60-MMP works only at relatively high dosage.

TABLE 2. Excretion of uranium in mice after repeated administration of DTPA and C60-MMP

Ligandsa	Dosages (mol/animal)	Excretion of U(VI) (mol/animal), n = 8
		Urineb
DTPA	0.11 × 3c	
C60-MMP	0.11 × 3c	

a Ligands were given to mice by IV injection of 0.1 mL uranyl nitrate.
b Excreta of each group were pooled and SD is not available.
c The 0.33 mol/animal was administered to mice in three injections, 0.11 mol/animal per injection.

It is reported that the most promising approach to chelation therapy for U(VI) appears to be combining ligands with different decorporation performances to gain access to U(VI) in kidney and bone. Indeed, there is a combination of

effective low-toxicity ligands that takes advantage of the greater ability of 5-LI(Me-3,2-HOPO) to chelate UO_2^{2+} in the kidneys and the greater potential of 5-LI-CAM(S) to chelate U(VI) already deposited in bone [**?**].

In this study, eight mice were subsequently treated with DTPA and C60-MMP after uranium nitrate injection. Results were compared with mice treated with equimolar amounts of either chelator alone. Figure 3 Figure 3: see original paper shows uranium distribution in blood and other target organs of mice receiving chelation therapy with combined ligands versus DTPA or C60-MMP alone. Unfortunately, the combination of DTPA and C60-MMP was found to be ineffective in eliminating uranium from all measured tissues and organs compared with mice treated with DTPA or C60-MMP alone. Instead, it significantly increased uranium retention in the kidney. As shown in Table 3 , little uranyl ion was detected in urine and feces due to high uranium deposition in kidneys of mice treated with combined drugs.

TABLE 3. Excretion of uranium in mice after injection of combined drug

Ligandsa	Excretion of U(VI) (mol/animal), n = 8
	Fecesb
DTPA	
C60-MMP	
DTPA+C60-MMPc	

a Ligands (0.1 mL) were given to mice by IV injection 30 min after IV injection of uranyl nitrate (0.1 mL).
b Excreta of each group were pooled and SD is not available.
c Mice were treated with 0.17 mol/animal of DTPA, then with 0.17 mol/animal of C60-MMP.

C. Efficacy of C60-MMP in the Treatment of Chronic Uranium Toxicity

Prolonged exposure to low uranium dosages can produce low-level or "subclinical" illness and other detrimental effects [**?**], and chronic uranium poisoning is more common in uranium applications. The therapeutic efficacy of C60-MMP in degrading chronic uranium poisoning was evaluated in this work. It should be noted that excreta were not collected because approximately 60% of UO_2^{2+} is excreted in urine within 48 hours.

Figure 4 [Figure 4: see original paper] shows total uranium activities retained in mouse blood and organs. In general, chelators including DTPA and C60-MMP were nearly ineffective in decorporating uranium from liver, kidney, and skeleton. Particularly, uranium retention in kidney and skeleton increased markedly in mice administered C60-MMP. Therefore, it can be concluded that C60-MMP is not suitable for chelation therapy of chronic uranium poisoning.

Conclusion

This study represents the first attempt to evaluate the decorporation efficacy of C60-MMP, a novel fullerene derivative, as a potential uranium removal ligand in mice. Experimental data supported that C60-MMP could efficiently prevent uranium deposition in livers during the first 8 hours and subsequently increase uranium excretion from livers. Additionally, C60-MMP helped accelerate uranium metabolism and reduce its retention in kidneys. Urine was found to be the main pathway for uranyl elimination, with C60-MMP-treated mice showing much more significant excretion than the other two groups. Unfortunately, investigations on the effects of repeated low-dose C60-MMP administration and combination with DTPA were almost ineffective in decreasing uranium deposition or urinary uranyl excretion. Moreover, C60-MMP is not recommended for use in chronic uranium intoxication.

Although C60-MMP is an efficient and novel uranium antidote, this research represents only the first step in a systematic approach to developing rational therapeutic protocols for uranium intoxication treatment. More efforts should be made to improve treatment protocols and clinical applications. For optimization of therapeutic efficacy, C60-MMP is advised to be combined with Tiron (as uranyl retention in bone is reported to be significantly reduced by Tiron in uranium removal in mice in the next survey [**?**]).

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Note: Figure translations are in progress. See original paper for figures.