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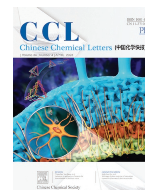

Supramolecular liquid barrier for sulfur mustard utilizing host-guest complexation of pillar[5]arene with triethylene oxide substituents

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A B S T R A C T

Sulfur mustard (SM) can be absorbed by skin quickly and cause serious system damage *via* reacting with nearly all cell constituents. Until now, there is still lack of effective antidotal therapy for SM and skin protection is highly important to defend SM. In this article, supramolecular liquid barrier based on pillar[5]arene with triethylene oxide substituents (EGP5) has been designed to impede the skin permeation of SM and further interaction with the skin tissue. EGP5 could encapsulate SM within its cavity, with a K_a value of $(5.10 \pm 0.47) \times 10^2$ L/mol. *In vitro* skin absorption test proved that EGP5 was capable to effectively prevent SM from penetrating through skin. This supramolecular liquid barrier was employed on rat models to systematically evaluate protective effect against SM intoxication. Pretreatment of EGP5 could alleviate skin and system damage induced by SM and improve survival rate of poisoned rat models from 10% to 90%. Additionally, EGP5 served as protective materials could be highly reused after recycling several times. Overall, these findings have provided the first insight into the construction of convenient liquid material for SM protection.

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Sulfur mustard (SM), a highly strong alkylating agent, is capable of reacting with DNA, proteins and other macromolecules, leading to inflammation, cellular apoptosis and necrosis [1–3]. At the organ level, skin is an important target tissue for SM due to sensitivity of frequently dividing basal cells. SM can be absorbed quickly through skin without any warning signals, inducing serious symptoms including erythema, blistering, ulceration of dermal structure [4–8]. In addition, SM could be redistributed into systemic circulation through depots formed in the lipid components of the skin, causing the systemic intoxication that involve the cardiovascular, central nervous and immune systems and even death of victims. Although the production, stockpiling and utilization of SM have been banned by the Chemical Weapons Convention in 1993 among more than 190 nations, the potential threat posed by SM is still relevant since such a simple molecule is easy-synthesized and full of charm for terrorist [9–11]. Research on this chemical weapon agent has been involved and continues to present day. However, its full clarification about toxic mechanism has not been proposed and there is still lack of effective antidotal therapy for SM exposure [12–14].

As a consequence, personal protection has been the last line of defense to impede contact with SM [15]. The most common forms of protective materials are solid porous absorbents. A typical one among them, activated carbon is restricted to serving as breath mask filters in many cases [16–18]. Moreover, considerable efforts have been devoted to explore novel protective materials, such as metal oxides, metal-organic frameworks and polyoxometalates, while these materials are reportedly non-selective, unstable and present issues for practical application [19–24]. Alternatively, encapsulation of SM using macrocyclic containers may help tackle the above-mentioned challenges.

Pillar[*n*]arene [25–30], with well-defined structure and excellent recognition properties was chosen as molecular scaffold. Recently, our group demonstrated that per-ethylated pillar[5]arene (EtP5) could form host-guest complex with SM and achieve absorption by activated EtP5 crystalline materials [31]. Although realizing efficient absorption, this system failed to apply in biosystem. In this article, supramolecular liquid barrier for SM was constructed for the first time. A liquid macrocycle, pillar[5]arene

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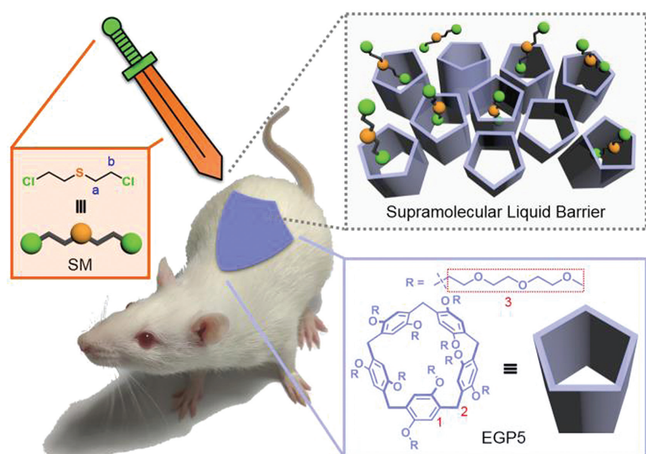

Fig. 1. Chemical structures of EGP5 and SM, and schematic illustration of EGP5 as supramolecular liquid barrier to protect against SM on rat model.

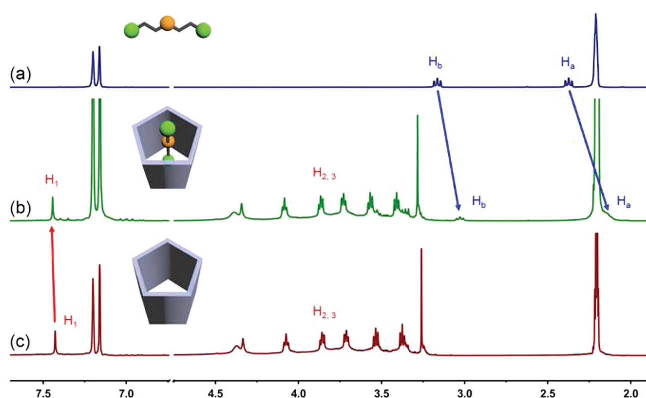

Fig. 2. ^{1}H NMR specta (400 MHz, *o*-xylene-d_{10}) of (a) SM (5.00 mmol/L), (b) EGP5 (5.00 mmol/L) with addition of SM (5.00 mmol/L) and (c) EGP5 (5.00 mmol/L).

with triethylene oxide substituents (EGP5) [32–35], could confine SM within its intrinsic cavity to achieve high protective efficiency against SM exposure (Fig. 1). Notably, macrocycle material at liquid state was convenient for personal usage without addition of solvent and provided flexibilities in meeting the demands for various equipment applications. NMR spectroscopy and fluorescence titration showed host-guest encapsulation of EGP5 towards SM. The protective effect of EGP5 against SM was performed on rat models. Liquid EGP5 could inhibit interactions between SM and skin tissue, thereby alleviating system damages and improving survival rate of poisoned rats. In addition, EGP5 possessed special lower critical solution temperature property and was capable to recycle over five times for reuse.

In this system, supramolecular liquid barrier is based on the host-guest interactions of SM. The complexation behavior between EGP5 and SM was first verified by ^{1}H NMR experiments. Fig. 2 showed the ^{1}H NMR spectra of SM in the absence and presence of 1 equiv. of EGP5. Upon the addition of EGP5, all the SM protons (H_a and H_b) exhibited remarkable upfield shifts ($\Delta\delta = -0.13$ and -0.20) and broadening effect of the SM protons (H_a) was considerably observed. Proton H_1 of EGP5 shifted downfield resulting from the complexation-induced de-shielding effect. To quantitatively assess the association constant (K_a) between EGP5 and SM, fluorescence titration was carried out. Upon addition of SM, the fluorescence intensity of EGP5 gradually increased, and the K_a value of SM/EGP5 was determined to be $(5.10 \pm 0.47) \times 10^{2}$ L/mol by a standard curve fitting protocol (Fig. S6 in Supporting information). Moreover, geometry optimization

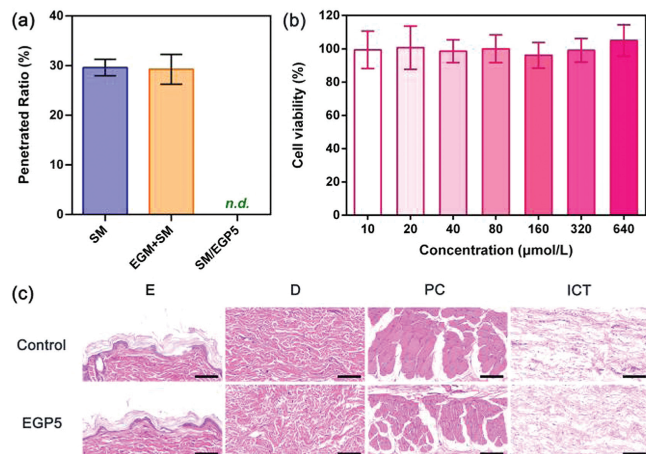

Fig. 3. (a) Penetrated ratio of SM through SD rat skin *in vitro* using a Franz-type diffusion cell system. Note: SM alone (0.72 µL) was applied in the donor cells in SM group, EGM+SM group and SM/EGP5 group were respectively pretreated with EGM (61.16 cm^{2}/mg) and EGP5 (63.69 cm^{2}/mg) before SM exposure. *n.d.* – no proton signals of SM were detected by ^{1}H NMR spectrum. (b) Cell viability of HaCaT cells treated with various concentrations (10–640 µmol/L) of EGP5 for 48 h. Cell viability was then measured using a CCK-8 assay ($n = 5$, mean ± SD). (c) H&E analyses of skin tissue from rat at 15 day after treatment with EGP5. E = epidermis, D = dermis, PC = Panniculus carnosus, and ICT = interstitial connective tissue. Normal rats served as control group (scale bar = 100 µm).

on SM/EGP5 was performed to obtain further evidence of the existence of 1:1 inclusion complex (Fig. S7 in Supporting information). For comparison, ^{1}H NMR of the mixture of macrocycle monomer (EGM) and SM was also conducted. Proton signals of both molecules exhibited negligible shift, demonstrating that EGM could not bind with SM (Fig. S8 in Supporting information).

In order to assess the barrier effect of EGP5 on the skin penetration of SM, the absorption experiment was performed *in vitro* using a Franz-type diffusion cell system. Tris(trimethylsilyl)phosphate (TMSP) was employed as referencing and relative SM amount was calculated according to ^{1}H NMR results (Fig. S9 in Supporting information). As shown in Fig. 3a, penetrated ratio of SM was up to 29.61% at 12 h after exposure, which is in accordance with previously reported results [37]. Pretreatment with control compound EGM led to no influence on skin absorption of SM with a penetration ratio of 29.25%. Notably, SM could not be detected in receptor chamber when EGP5 was applied on skin before SM exposure, leading us to suggest that EGP5 could serve as protective barrier to inhibit skin penetration.

Prior to investigation of protective efficiency *in vivo*, safety profile of EGP5 was evaluated. The cell viability of EGP5 in human immortalized keratinocytes (HaCaT) was assessed first using a Cell Counting Kit-8 (CCK-8) assay. As shown in Fig. 3b, minimal cytotoxicity of EGP5 was observed over the testing range. The change in rat body weight was monitored as another evidence for system toxicity. Similar bodyweight growth curve of EGP5 group could be seen, compared to that of control group (Fig. S10 in Supporting information). No skin allergic responses were observed and further support for low toxicity inferred for EGP5 came from histological analysis of skin tissue. Compared with control group, no detectable lesions or histopathological abnormality could be noted in EGP5 group (Fig. 3c). Moreover, routine blood and blood biochemistry assays were also conducted and the data revealed that there was no discernable change in white blood cells (WBC), alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase (CK), triglyceride (TG) and lactate dehydrogenase (LDH) after employment with EGP5 (Fig. S11 in Supporting information). Taken together, above results led us to consider that EGP5 could

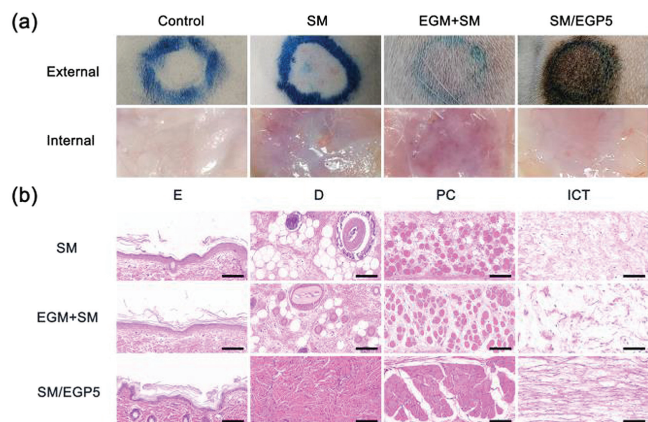

Fig. 4. (a) Macroscopic symptoms on rat skin of direct SM (2 µL) poisoning group, EGM (61.16 cm^2/mg, EGM/SM = 5:1) and EGP5 (63.69 cm^2/mg, EGP5/SM = 1:1) protective groups. (b) H&E stains of skin tissue excised after the indicated treatments. E = epidermis, D = dermis, PC = Panniculus carnosus, and ICT = interstitial connective tissue (scale bar = 100 µm).

be applied safely as supramolecular barrier to proceed *in vivo* protective evaluation.

As noted in the introduction, SM can be absorbed quickly through skin and cause serious system damage. Thus the protective efficacy of liquid EGP5 was verified on SM poisoning rat model. Direct exposure to SM (2 µL), blisters and erythema, features that characteristic of SM intoxication remarkably occurred on the skin surface [36]. While pretreatment with macrocycle EGP5 (63.69 cm^2/mg) reduced the morphological changes induced by SM. For comparison, control monomer EGM (61.16 cm^2/mg) had no effect on the skin symptom of SM poisoning. Moreover, the damage of deeper subcutaneous layer in SM and EGM+SM group was encountered, including large white edema and dark red appearance. EGP5 could apparently alleviate the subcutaneous layer lesion (Fig. 4a).

Hematoxylin and eosin (H&E) was also performed to evaluate skin damage (Fig. 4b). Compared with control group, SM poisoning led to epidermal necrosis and dermal vesicles. Muscle fiber in the panniculus carnosus even experienced deformation and there was abnormal observation of red blood cells. EGM pretreatment was ineffective against SM skin toxication and showed the similar tissue lesion as SM group. However, rats pretreated with EGP5 exhibited alleviation of skin damage and the tissue structure was intact without vesiculation and deformation of collagen fiber and muscle fiber.

In view of above exciting outcomes, we thus set out to test whether this liquid protective material could provide a benefit in survival rate of SM poisoned rats. Kaplan-Meier analyses showed that SD rats with direct SM treatment occurred a mortality rate of 90% on day 5, demonstrating the lethal effect of this dose of exposure. The survival curve of EGM + SM group is similar to that of the SM group (Fig. 5a). Remarkably, pretreatment with EGP5 significantly improved survival rate and 90% poisoned rats were successfully alive on day 8. In addition to get better understanding of the EGP5 protective efficiency, the impact of diverse groups upon system damage was studied by collecting blood samples at 6 h. ELISA assay was carried out to assess serum level of TNF-α and IL-6 in each group. Firstly, the appropriate calibration curves of TNF-α and IL-6 were derived (Figs. S12 and S13 in Supporting information). Compared with control group, the expression of TNF-α and IL-6 in SM and EGM + SM group were significantly increased. On the contrary, there were no obviously inflammation factors increasement by pretreating with EGP5 (Figs. 5b and c). Furthermore, sharp decrease of WBC level was observed in SM group, which was consistent to previous research results [38]. WBC level

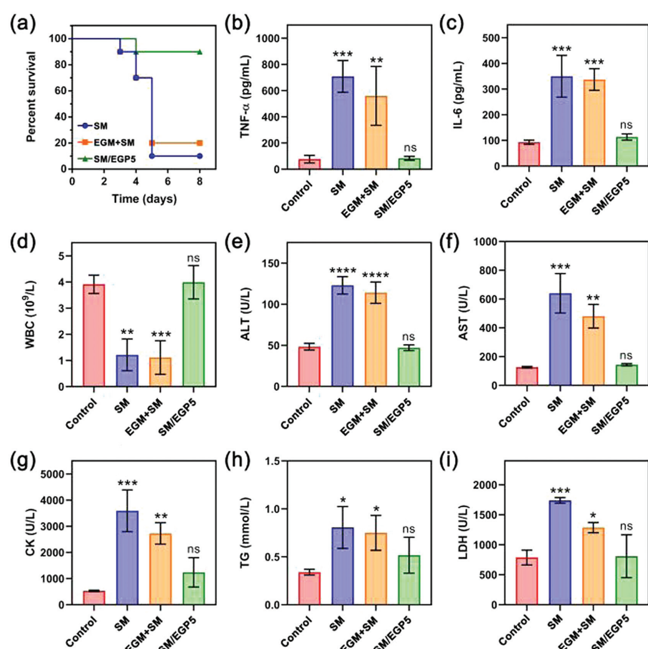

Fig. 5. (a) Survival rate of SM poisoning rats in several groups (n = 10). (b) TNF-α and (c) IL-6 levels in rat serum were assessed using ELISA method. (d) WBC, (e) ALT, (f) AST, (g) CK, (h) LDH and (i) TG levels after 6 h post cutaneous exposure to SM. Untreated normal rat served as control group. Data in (d)~(k) are represented as mean ± SD, n = 3. P-values are determined using one-way analysis of variance test. ns, no significance. $^{*}P \le 0.05$, $^{**}P \le 0.01$, $^{***}P \le 0.001$ and $^{****}P \le 0.0001$.

of rats pretreated with EGP5 had no change compared to control group, indicating that EGP5 successfully blocked the inflammation caused by SM (Fig. 5d). Blood biochemistry assays further proved that EGP5 could significantly alleviate the abnormal changes in the levels of ALT, AST, CK, TG and LDH caused by SM poisoning (Figs. 5e-i). These results were taken as further evidence that EGP5 formed supramolecular liquid barrier was effective enough to relieve SM-induced inflammation and tissue damage in main organs.

Subsequently, the back skin tissue of poisoned rats in each group were excised respectively after 12 h and 15 day of SM exposure. After removing subcutaneous fat, fresh skin was placed in deuterium solution. Following ultrasound and separation process, SM in extraction solution was monitored by ^{1}H NMR spectrum (Figs. S14 and S15 in Supporting information). Without pretreatment with EGP5, no detectable proton signals relative to SM could be measured at 12 h. While characteristic peaks of SM could be obviously observed in SM/EGP5 group and SM protons underwent upfield shift, as a consequence of the inclusion-induced shield effect. Interestingly, SM signals still could be measured at 15 day under the condition of existence of EGP5 formed supramolecular barrier. Taken together, these favorable findings indicated that EGP5 could efficiently complex with SM *via* host-guest interactions to inhibit its skin penetration, thereby exerting protective function.

Protective materials against SM were hard to achieve recyclability as a result of fouling, however recycling performance was an important parameter in practice applications. We succeeded in using a liquid-liquid extraction method to isolate EGP5. Water and petroleum ether served as extraction solution and EGP5 could totally dispersed into aqueous phase. Moreover, benefiting from the combination of hydrophilic tri(ethylene oxide) moieties and hydrophobic pillar[5]arene backbone, EGP5 performed a lower critical solution temperature behavior that could aggregate at the temperature above clouding point (50 °C). Inspired by this, we obtained EGP5 from aqueous phrase by heating and separation process. ^{1}H NMR spectra showed that EGP5 sample was

considered as pure since no significant contaminant was observed (Fig. S16 in Supporting information). And the recycle percentage (84.5%) after recycling five times was satisfactory (Fig. S17 in Supporting information).

In summary, we reported a supramolecular liquid barrier for SM for the first time. Liquid macrocycle EGP5 could effectively capture SM to inhibit its skin penetration *via* host-guest interactions with a K_a value of $(5.10 \pm 0.47) \times 10^2$ L/mol. The protective effect was actually validated by SD poisoning model. Pretreatment with EGP5 significantly alleviated system damage and decreased the mortality rate of poisoned rats. Notably, EGP5 could be recycled by simple methods over five times. This discovery provides an important foundation to support the potential application of EGP5 as supramolecular liquid barrier for protection against SM. It will also open up a new avenue that such a strategy can be extended to protect against other skin corrosive agents and provide a new insight for development of liquid functional materials.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.07.040.

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