Original

The Maximum Levels and Distribution of Volatilized Cyclophosphamide Gas in Air

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Abstract

Background: Cyclophosphamide was developed from nitrogen mustard and has been used as an alkylating anticancer drug. Gasification of cyclophosphamide has been suspected to occur, and the use of closed systems for cyclophosphamide has been preferred in mixing for preventing occupational exposure. However, previous reports on the levels of cyclophosphamide in air have been conflicting.

Methods: In order to determine the maximum levels of gaseous cyclophosphamide, the volatilization chamber which includes the box with the remote-controlled ceiling was placed at respective temperature. The distribution of gaseous cyclophosphamide was also investigated using the chamber without fanning the air.

Results: The maximum levels of cyclophosphamide gas at 23°C were 0.99 ng/mL, which suggested the upper risk limits of exposure to healthcare workers. As for the distribution of gaseous cyclophosphamide, the levels at the lower and middle layer of the chamber were height-dependent and the level at the upper layer was below the detection limit.

Conclusion: This study showed the maximum levels at respective temperature and the distribution of gaseous cyclophosphamide in air. These results will contribute for preventing occupational exposure to cyclophosphamide.

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—Key words—

cyclophosphamide, occupational exposure, gasification

Background

Hazardous chemicals such as anticancer drugs are regarded as carcinogenic and teratogenic, and can cause reproductive toxicity, genotoxicity, and organ toxicity[1)]. Healthcare workers who handle hazardous drugs are at a high risk of occupational exposure to these drugs. These drugs have been detected in the urine of healthcare workers handling them, indicating that the drugs are absorbed into the human body[2)3)]. The increased health risk to the staff that handles hazardous drugs was shown in some reports, two of which revealed that the rate of spontaneous abortion in healthcare personnel who handled hazardous drugs was elevated compared to that in the control group[4)5)].

Cyclophosphamide is an alkylating anticancer drug often used to treat breast cancer and malignant lymphoma. It was originally developed from nitrogen mustard, a derivative of mustard gas known for its use as a chemical warfare agent in World War 1[6)]. Owing to its historical background and similar chemical structure, gasification of cyclophosphamide has long been suspected to occur. In addition, the use of closed systems for anticancer drugs such as cyclophosphamide has been highly preferred due to the potential gasification.

However, reports on the gasification of cyclophosphamide have been conflicting. Connor et al. designed an experiment using the Ames test, which evaluates the genotoxicity of agents using reverse mutation in bacteria, and qualitatively revealed gasification of cyclophosphamide[7)]. The reported levels of gaseous cyclophos-

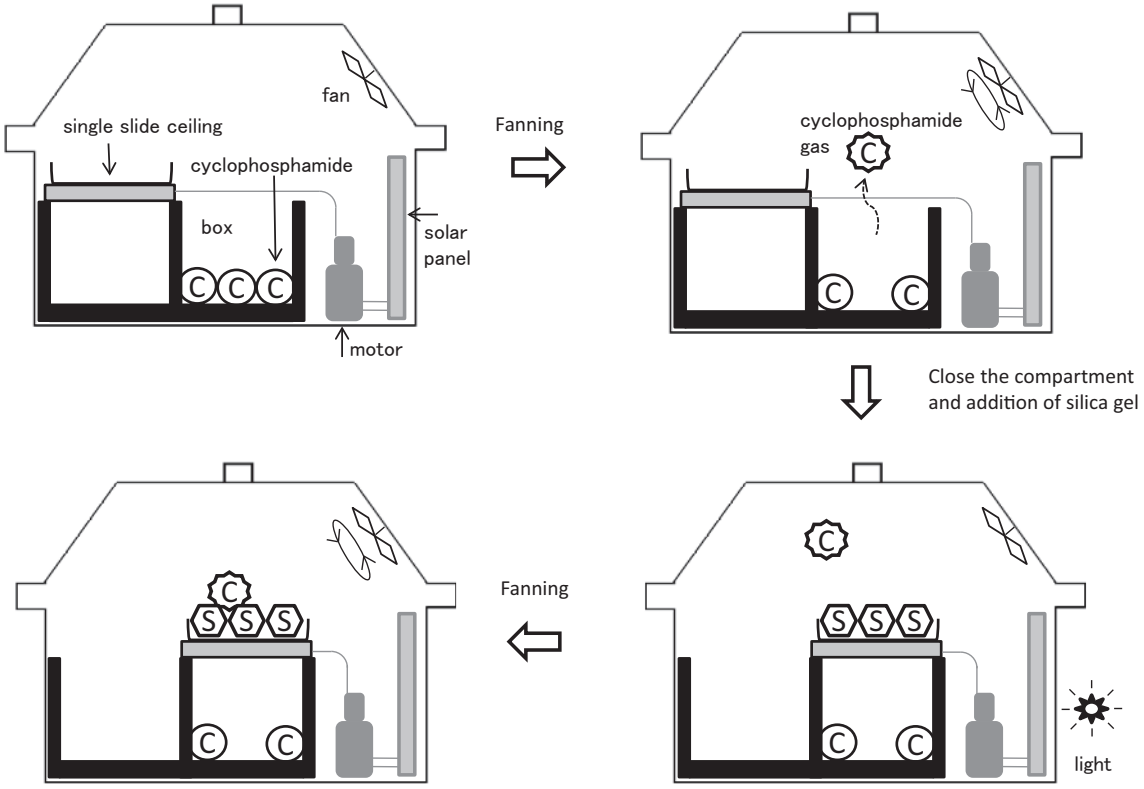

Fig. 1 Illustration of the procedure for determination of the levels of volatilized cyclophosphamide

phamide differed by approximately three orders of magnitude between the other reports[8)9)].

Therefore, the aim of our study was to analyze gasification of cyclophosphamide quantitatively and to resolve the confusion presented by the earlier reports. We also studied the distinct distribution of cyclophosphamide gas in air.

Materials and Methods

Reagents

Endoxan containing 500 mg of cyclophosphamide powder was purchased from Shionogi & Co., Ltd (Japan). A silica gel tube (Gastec 252S-20, Japan) was opened and prepared for cyclophosphamide gas extraction. MonoTrap RSC18 was obtained from GL Sciences (Japan).

Experimental chamber for maximum volatilization

The volatilization chamber of 0.21 m tall was prepared as illustrated in Fig. 1. The box which was divided into two compartments was placed in the volatilization chamber. The single sliding ceiling of the box was fastened to the motor (Solar motor 03, Tamiya, Japan) outside the box by the line. Since the motor was hooked up to the solar panel (0.5 V−1,500 mA, Tamiya, Japan), the single slide ceiling of the box can be moved by the fluorescent lamp (15 W) outside the volatilization chamber. The air in the chamber was agitated by a fan (Kawashima Seisakusho, Japan). The chamber was placed in a temperature-controlled incubator and the inner temperature of the chamber was monitored by thermometer (Omron, Japan).

Volatilization and absorption of cyclophosphamide gas

Cyclophosphamide powder (500 mg) was placed in one compartment of the box and the single slide ceiling was pushed to the other side. The fan was turned on to induce volatilization of cyclophosphamide. After the appropriate periods of time, the single sliding ceiling was moved by the fluorescent lamp to close the compartment which contained cyclophosphamide, and the silica gel was added to the chamber. The silica gel was recovered after 10 min of cyclophosphamide gas absorption.

Determination of cyclophosphamide gas

The cyclophosphamide in the silica gel was extracted with methanol for subsequent LC/MS/MS analyses,

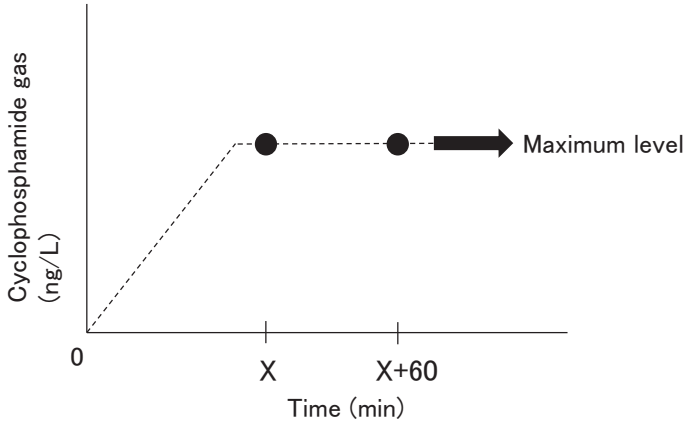

Fig. 2 Maximum level of cyclophosphamide gas. The levels at the two time points separated for 60 min (X and X + 60 min) should be almost the same, after the levels of volatilized cyclophosphamide have reached the maximum.

which were originally established. Determination was carried out using an Ultimate 3000 series high-performance liquid chromatography system, which includes a HPG-3400RS pump, a WPS-3000TRS autosampler, a TCC-3000RS thermostat column compartment, and a triple quadrupole mass spectrometer TSQ Endura equipped with an electrospray ionization source (ThermoFisher Scientific, MA, USA). Separation was performed on an Acclaim RSLC 120 C18 column (2.2 μm, 2.1 × 100 mm) with the column temperature maintained at 40℃. The sample injection volume was 1 μL. The mobile phase consisted of a mixture of phase A (ultrapure water) and phase B (acetonitrile) with a flow rate of 0.6 mL/min. The gradient program was set as follows: 0–1.5 min, 10% B; 1.5–4.0 min, 10–60% B; 4.0–4.2 min, 60–90% B; 4.2–6.0 min 90% B; 6.0–6.2 min 90–10% B; 6.2–8.0 min 10% B.

Quantification was performed using electrospray in the positive mode with the spray voltage set at 3,500 V. Nitrogen was used as the sheath gas. Argon gas was used as the collision-induced gas, with pressure set at 3 mTorr. The temperature of the ion transfer tube and vaporizer were 250℃ and 400℃, respectively. RF lens and source fragmentation were set at 150 V and 10 V, respectively. The multiple reaction monitoring transitions m/z 261→106.3, 261→140.09 and 261→141.96 were selected, whose collision energy were 19.5, 24.3 and 17.7 V, respectively. The detection limit of cyclophosphamide was 0.1 pg.

Maximum level of cyclophosphamide gas

The maximum level of cyclophosphamide gas had to satisfy the following two conditions: 1) The temperature inside the chamber has reached the target temperature; 2) the levels of the cyclophosphamide gas were almost equal at two points separated for 60 min (Fig. 2).

Distribution of cyclophosphamide gas

The experimental chamber for the distribution analysis of cyclophosphamide gas was as illustrated in Fig. 3. Cyclophosphamide was placed in the bottom of the chamber. Then, Monotrap RSC18 (GL Sciences, Japan) was attached by a string at the upper layer (0.2 m from the bottom), middle layer (0.1 m), or lower layer (0 m) of the chamber. After a 24 hr incubation, Monotrap RSC18 was recovered and treated with methanol. The absorbed cyclophosphamide was extracted and determined by LC/MS/MS as described above.

Results

The levels of cyclophosphamide gas at 23°C on time 0 and 60 were 0.92 and 1.06 ng/mL, respectively, which were considered the maximum value. The mean was 0.99 ng/mL (Table 1). The mean levels of cyclophosphamide gas increased as the temperature inside the chamber increased from 23 to 50°C. The level of cyclophosphamide gas at 50°C was 5.36 ng/mL, which was approximately 5 times greater than the level at 23°C.

In order to obtain reproducible results in the experiments described above, it is necessary to fan the air in the chamber. This suggested that the distribution of cyclophosphamide gas was not uniform. Therefore, we

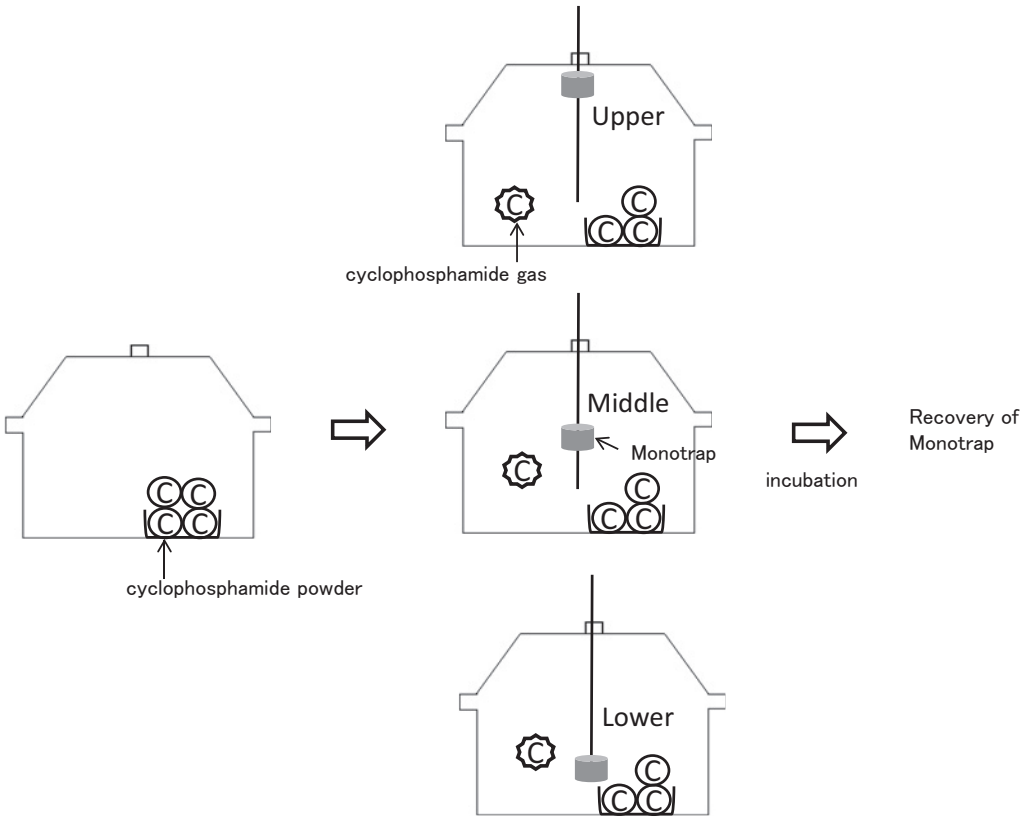

Fig. 3 Illustration of the procedure for determination of the distribution of volatilized cyclophosphamide

Table 1 The levels of cyclophosphamide gas at various temperatures

Temperature (℃)	Cyclophosphamide gas (ng/L)		
	Time after reaching to the target temperature (min)		Mean
	0	60	
23	0.92	1.06	0.99
30	2.62	1.64	2.13
40	3.03	2.46	2.75
50	5.27	5.44	5.36

decided to examine the distribution of cyclophosphamide gas. The levels of cyclophosphamide gas at the lower and middle layer of the chamber were 0.26 and 0.16 ng/mL, respectively and the level at the upper layer was below the detection limit (Table 2). The levels of cyclophosphamide gas progressively decreased as the sampling points of the gas became higher.

Discussion

The first report on vaporization of cyclophosphamide, published by Connor et al., showed that cyclophosphamide vaporized from dishes and exhibited reverse mutation activity in the Ames test[7]. The quantitative analysis regarding the drug studied by Nakanishi et al. showed that vaporized cyclophosphamide was detected at 40 and 30℃, but not at 25 or 20℃[8]. Sato et al. also reported levels of cyclophosphamide in air after vaporization at 35–40℃[9]. These frontier experiments were similar in their assertion that cyclophosphamide was absorbed to the adsorbent while it continued to vaporize, making it difficult to determine the saturated vaporization level of the drug in air. In addition, the levels mentioned in the reports varied by approximately three orders of magnitude, although the experimental details differed.

The maximum levels of cyclophosphamide in air determined in our study were between 0.99 and 5.36 ng/L, which suggested the upper risk limit of exposure to the drug in healthcare workers including pharmacists.

Table 2 The distribution of cyclophosphamide gas

Position: distance from the bottom	Cyclophosphamide gas (ng/L)
Upper layer: 0.2 m	N.D.
Middle layer: 0.1 m	0.16
Lower layer: 0 m	0.26

These values are consistent with those reported by Sato et al[9]. Our results also revealed that the levels of the drug were temperature-dependent, which is consistent with the report by Nakanishi et al[8]. The regression line of the maximum volatilized levels of cyclophosphamide was almost linear in the studied temperature range.

We recognized the need for the fan in our study, since the reproducibility was not satisfactory without fanning. This observation led us to the hypothesis that the distribution of volatilized cyclophosphamide gas is not uniform—it remains at a lower height, closer to the powder surface. The levels of cyclophosphamide were height-dependent and not detected at the highest position in the chamber without fanning. Historically, cyclophosphamide was developed from nitrogen mustard, which was the derivative of the chemical warfare agent, mustard gas[6]. It is understandable, then, that the poisonous gas remains lower near the ground to exert its toxic effects. The molecular weight of cyclophosphamide is 279.1 and approximately ten times larger than that of air, 28.8. Since methane gas, a major component of city gas, ascends and propane gas descends in air, cyclophosphamide gas would remain in the lower layers of the atmosphere. These facts may lend support to our observation that fanning was required to sustain uniform distribution of cyclophosphamide gas in air.

The distribution of cyclophosphamide gas in the lower layer leads us to the question of whether cyclophosphamide gas could possibly be inhaled by healthcare personnel. Based on our findings, volatilized cyclophosphamide takes time to ascend to the levels at which the healthcare professionals can breathe, since it continues to spread on surface if the air is not agitated uniformly. Our data also suggest that biological safety cabinets that exhaust air pulled through dedicated exhaust ducts are desirable to prevent human exposure to the gas.

The dose-response effects with respect to the exposure levels of the anticancer drugs remain obscure. In addition, healthcare professionals can be exposed to anticancer drugs via splash, spill, and volatilization. Although we revealed the maximum concentration and distribution of volatilized cyclophosphamide gas, further studies are needed to evaluate the exposure risk for healthcare workers who are involved in preparing cyclophosphamide.

Conclusion

Our study revealed the levels at respective temperature and the distribution of gaseous cyclophosphamide in air. These results will contribute for preventing occupational exposure to cyclophosphamide.

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シクロホスファミドの最大気化量と分布に関する研究

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—キーワード—
シクロホスファミド，職業曝露，ガス化

背景：シクロホスファミドはナイトロジェンマスタードから開発され，アルキル化抗がん剤として使用されている．シクロホスファミドはガス化することが疑われており，職業曝露を防ぐために注射薬調製時には閉鎖式混合調製器具の使用が推奨されている．しかし，これまで空気中のシクロホスファミド量に関する報告は相反していた．

方法：シクロホスファミドの最大気化量を測定するため，外部から天井の移動をコントロールできる小箱が入った気化箱を各温度に置いた．シクロホスファミドガスの分布は，ファンを中で回していない気化箱を使って調べた．

結果：シクロホスファミドの最大気化量は 23℃ で 0.99ng/mL であり，この値は医療従事者が被ばくしうる上限値と考えられる．シクロホスファミドの分布に関しては，気化室の下層と中層では高さ依存的であったが，上層では検出限界以下であった．

結論：私たちの研究は各温度下でのシクロホスファミドの最大気化量および分布を示したものである．これらの結果はシクロホスファミドの職業曝露を防ぐために寄与するであろう．

［COI 開示］本論文に関して開示すべき COI 状態はない．

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