—Regular Article—

Light-induced Deterioration Test of Carboplatin under Clinical Settings

Yoshiaki OKAMOTO,*,[a] Keiko TAZUMI,[b] Yasuaki SANADA,[a]
Mamiko TSUGANE,[a] and Etsuko UEJIMA[a]

[a]*Department of Hospital Pharmacy Education, Graduate School of Pharmaceutical Science, Osaka University, 1–6 Yamadaoka, Suita, Osaka 565–0871, Japan, and* [b]Chemotherapy and Oncology Center, Osaka University Hospital, 2–15 Yamadaoka, Suita, Osaka 565–0871, Japan

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Chemotherapeutic drug dosages are calculated precisely based on the patient's height, body weight, and renal function, *etc*. To ensure safe and favorable outcomes of treatment, dosing solutions are prepared by appropriate mixing of the drug solutions based on such calculations. The package inserts for many injectable preparations include a warning for storing the product ''shielded from light.'' However, there are no reports of stability assessment of a mixed product against light exposure or the residual amount of active ingredient in the dosing solution during or at the end of treatment. We evaluated the stability of carboplatin from the time of mixing of the dosing solution until the end of drug infusion in a clinical-like setting. With 4-hour exposure to outdoor scattered light, the dosing solution began to show discoloration by 1 hour, becoming dark yellow by 4 hours, with reduction of the percent residual carboplatin to about 23%. To identify the optimal light-shielding shade, the dosing solution was shielded from outdoor scattered light with 1 of 3 protective covers: aluminum foil, yellow plastic shade, and brown plastic shade. The yellow plastic shade prevented any changes of the appearance of the dosing solution during the 4-hour exposure period. The percent residual carboplatin, determined by HPLC, in the dosing solution shielded with a yellow plastic shade was about 85.2% at 2 hours and 78.6% at 4 hours. Thus carboplatin dosing solution should be completely shielded from light until infusion is completed.

Key words——deterioration test; light shielding; chemotherapeutic drug; carboplatin

INTRODUCTION

Chemotherapeutic drug dosages are usually calculated precisely on the basis of the patient's height, body weight, and general condition (renal function, *etc*.). The dosing solution is prepared by appropriate mixing of drug solutions based on such calculation to ensure safe and favorable outcomes of treatment. The package inserts for many injectable preparations include a statement warning that the product should be stored in light-shielding condition. However, it is uncommon for data or statements about the stability of a product against light exposure after mixing to be provided in the package insert. Instability of products against light exposure has also been reported.[1–4] Preparations of vitamin-supplemented IV solutions for hyperalimentation, designed for 24-hour serial infusion, are usually administered while covered with a specific light-shielding shade. However, long-term serial infusion of chemotherapeutic agents is sometimes carried out without using any light-shield cover, because no specific light-shielding cover for such preparations is available.

As the number of cases requiring pharmacists' guidance on medication management has increased, pharmacists now have greater opportunities to be in attendance at the time of administration of chemotherapeutic agents to patients. At such occasions, chemotherapeutic agents are often placed without light-shielding. Reduction in the potency of chemotherapeutic agents is directly associated with their therapeutic efficacy; therefore this parameter affects survival of the patients. Furthermore, it is possible that adverse reactions may arise due to degradation of the drug by ultraviolet rays, *etc*. In recent years, the number of outpatients receiving chemotherapy has increased; and treatment facilities often have large windows to allow adequate lighting, based on consideration of the patients' psychological aspects.

Carboplatin is a chemotherapeutic agent that is often used clinically and its package insert includes a warning that the product should be stored shielded from light. This agent was thus selected in the present study, to evaluate its stability against light exposure during the period from the time of mixing the solution for injection to the end of drug infusion under

*e-mail: okamoysh@phs.osaka-u.ac.jp

clinical settings, with the cooperation of the staff of an outpatient chemotherapy room. The duration of light exposure was set at a maximum of 4 hours, since the time needed for administration at our facility was most frequently 3 hours.

METHODS

Evaluation of the Necessity of Light Shielding First, the necessity of shielding from light was evaluated. To this end, carboplatin solution contained in a brown light-shielding shade (designed for a parenteral hyperalimentation solution) and in an IV solution bag not shielded from light was exposed to direct sunlight outdoors, and the changes in the external appearance and the percent residual carboplatin were examined.

Preparation of the Reagents Carboplatin (Paraplatin®, Bristol) was mixed with 500 ml of physiological saline (NaCl 0.9%) at a concentration of 450 mg/45 ml. Of the 2 samples prepared, one was placed in a bottle covered with a brown light-shielding shade designed for a parenteral hyperalimentation solution and the other was placed in a bottle covered with no shield. Both samples, prepared indoors at the same time, were immediately subjected to the outdoor test.

Appearance After the test samples were prepared indoors, they were exposed to outdoor light, and changes in their external appearance were examined macroscopically at the start and at 1 hour and 4 hours after the start of the test.

Illuminance The illuminance during exposure to scattered light and that within each light-shielding shade were measured at the start and at 1 hour and 4 hours after the start of the test, using an Illuminance Meter IM-5 (Topcon, Tokyo, Japan).

Measurement of Percent Residual Carboplatin A portion of the solution from each of the test packs was sampled at 1 hour and 4 hours after preparation of the solutions for the tests, as shown below.[5,6]

Conditions for HPLC The concentration of carboplatin was determined by reverse-phase liquid chromatography on Waters Acquity Ultra Performance LC system (Waters, Milford, MA, USA) equipped with an Acquity UPLC BEH C18 column (100 mm × 2.1 mm, i.d., 1.7 μm particle size, Waters) and a photo diode array detector (Waters). Elution was performed isocratically, with the mobile phase consisting of 3.3 mM tetrabutylammonium hydrogen sulfate (pH 7.5) and acetonitrile (89 : 1). The system was operated at 25°C and a flow rate of 0.3 ml/min and the injection volume was 2 μl. The detection wavelength was set at 230 nm. The amounts of carboplatin were quantified from the integrals of the peak areas.

Evaluation of the Effects of the Light-shielding Shade Then, the differences in the effects among different types of light-shielding shades were analyzed. Carboplatin samples in 3 containers supplied by the manufacturer as containers for a parenteral hyperalimentation solution covered with a brown shade, yellow shade, or aluminum foil were exposed to outdoor light, and changes in the external appearance and percent residual carboplatin were investigated over time, to identify the optimal dosing conditions for carboplatin.

Preparation of the Reagents Carboplatin (Paraplatin®, Bristol) was mixed with 500 ml of physiological saline (NaCl 0.9%) at a concentration of 450 mg/45 ml. The samples prepared were placed in containers covered with 1 of 3 light-shielding shades each (brown shade, yellow shade, and aluminum foil). These samples, prepared indoors at the same time, were immediately subjected to the outdoor test.

Appearance After the test samples were prepared indoors, they were exposed to outdoor light, and changes in their external appearance were checked macroscopically at the start and at 1, 2, and 4 hours after the start of the test.

Illuminance The illuminance during exposure to scattered light and that within each light-shielding shade were measured at the start and 1 and 4 hours after the start of the test, using an Illuminance Meter IM-5 (Topcon, Tokyo, Japan).

Measurement of Percent Residual Carboplatin Percent residual carboplatin was measured at 1, 2, and 4 hours after the start of the test by HPLC under conditions identical to those described above.

Measurement of Illuminance in Wards and Outpatient Chemotherapy Room Illuminance at the window of the wards and the outpatient chemotherapy room of our hospital was measured on a day of fine weather. Using an Illuminance Meter IM-5 (Topcon, Tokyo, Japan), identical to the device used in the above experiments, the mean and standard error of illuminance at 4 points near the window were calculated.

Statistics All statistical analyses were per-

formed using Graphpad Prism software (San Diego, CA). Differences between two groups were analyzed by Student's *t*-test. Differences among >3 groups were analyzed by one-way analysis of variance (ANOVA) with post hoc Bonferroni's test.

RESULTS

Necessity of Light Shielding

Illuminance and Changes in External Appearance The mean illuminance during the exposure period to outdoor light was 75.23±0.32 kLux. The samples within containers not shielded from light underwent a change of color to light yellow by 1 hour after the start of exposure. The samples within containers covered by the brown shade showed no change in external appearance (Table 1).

Percent Residual Carboplatin Representative HPLC chromatograms of carboplatin solution contained in the brown shade and in the non-light-shielded shade are shown in Fig. 1. The retention time for carboplatin was found to be 1.8 min. Four degradation products of carboplatin appeared under both conditions and the retention time of these products was 0.7, 0.8, 1.1, and 2.3 min, respectively.

When the HPLC charts at 4 hours were compared, the carboplatin peak area was smaller for the non-light-shielded samples than for the light-shielded samples and the area of the other peaks was greater in the non-light-shielded samples (Fig. 1).

The percent residual carboplatin in the non-light-shielded samples was as low as 22.7% at 4 hours, while no marked change in the amount of carboplatin was noted in the samples within containers covered by the brown shade (percent residual carboplatin at 4 hours, 93.2%) (Fig. 2).

Effect of Light-shielding Shade

Changes in External Appearance No change in external appearance was noted during the 4-hour period in any of the solutions within containers covered with the brown shade, yellow shade, or aluminum foil (Table 2).

Table 1. Changes in the Appearance of the Carboplatin Dosing Solution

Storage condition	0	1 h	4 h
Illuminance (kLux)	75.00	75.10	75.60
plastic brown shade (diffuse light)	colorless fluid	no change	no change
no shade (sunlight)	colorless fluid	light yellow	dark yellow

Illuminance The mean illuminance during the exposure period to scattered light was 48.14±6.68 kLux.

Inside the container shielded with the brown shade, the mean illuminance was 7.98±1.88 kLux; the light-cutting rate was 83.4%. Inside the container shielded with the yellow shade, the mean illuminance was 13.82±1.71 kLux; the light-cutting rate was 71.3%. Inside the container shielded with the aluminum foil, the mean illuminance was 0.20±0.12 kLux; the light-cutting rate was 99.6% (Table 2).

Percent Residual Carboplatin The percent residual carboplatin in the saline-diluted carboplatin solution after exposure to outdoor light was analyzed (relative to the concentration at the start=100%). Four hours after the start of exposure, the percent residual carboplatin in the containers covered with the aluminum foil and the brown shade was 99.7% and 97.4%, respectively, and was significantly lower in the container covered with the yellow shade (78.6

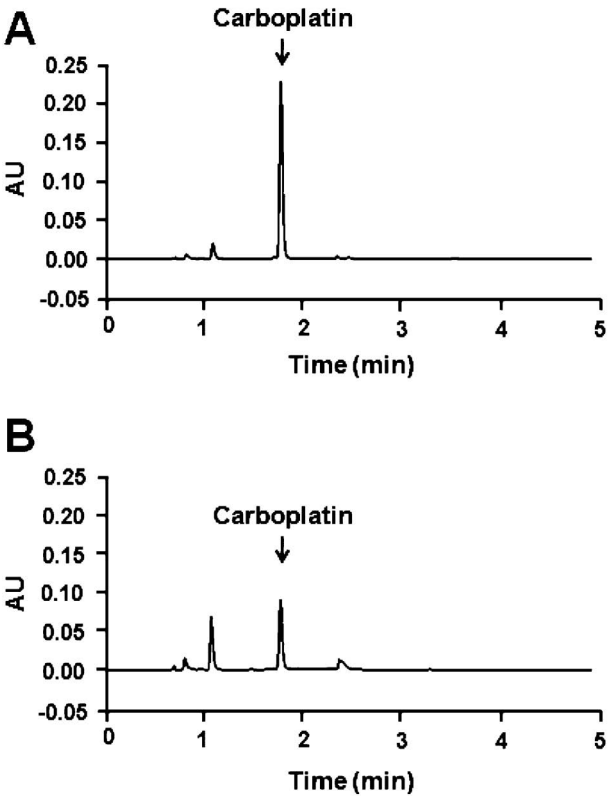

Fig. 1. Representative HPLC Chromatogram of Carboplatin at 4 hours
A: plastic brown shade, B: no shade.

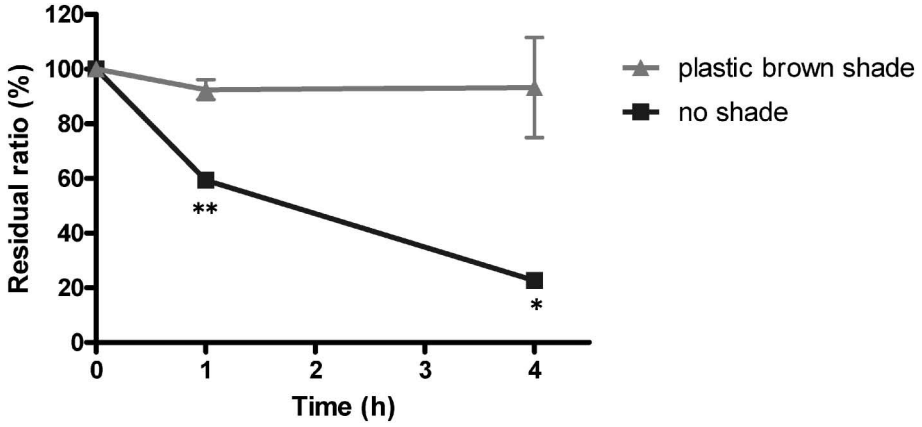

Fig. 2. Percent Residual Carboplatin in Dosing Solutions (Plastic Brown Shade *vs.* No Shade)
Data are presented as mean±S.E.M. ($n=3$). *$p<0.05$, **$p<0.01$ by Student's t test.

Table 2. Percent Residual Carboplatin in the Dosing Solutions

Storage condition	Hours after preparation	0	1 h	2 h	4 h
sunlight	Illuminance (kLux)	54.57	52.99	44.14	40.85
plastic brown shade (diffuse light)	appearance	colorless fluid	no change	no change	no change
	Carboplatin (%)	100	98.5	98.0	97.4
	Illuminance (kLux)	9.31	6.65	7.73	7.70
plastic yellow shade (light permeable)	appearance	colorless fluid	no change	no change	no change
	Carboplatin (%)	100	95.9	85.2	78.6
	Illuminance (kLux)	12.61	22.70	15.03	20.60
aluminum foil (lightproof)	appearance	colorless fluid	no change	no change	no change
	Carboplatin (%)	100	100.2	99.9	99.7
	Illuminance (kLux)	0.17	0.15	0.09	0.37

%) (Table 2 and Fig. 3). The percent residual carboplatin differed significantly between the containers covered with the aluminum foil and the yellow shade ($p<0.001$), between the containers covered with the aluminum foil and the brown shade ($p<0.01$), and between the containers covered with the yellow shade and the brown shade ($p<0.001$) at 1, 2, and 4 hours after the start of exposure.

Illuminance in Wards and Outpatient Chemotherapy Room The illuminance on a fine weather day was 59.85±6.67 kLux in the wards and 44.27±12.04 kLux in the outpatient chemotherapy room.

DISCUSSION

The dosage of carboplatin is usually calculated using the equation of Calvert based on renal function level in individual patients[7] rather than on the basis of the body surface area. This method of dosage calculation requires blood sampling from the patient and, in some cases, 24-hour pooled urine. Many clinical studies have been carried out to determine the efficacy and adverse effects of carboplatin administered at dosages calculated in this manner, allowing determination of the optimum dosage of this drug depending on the indication.[8–11] To enable administration of carboplatin precisely at the dosage calculated, thus accurate adjustment of the dosing mixture needs to be carried out as well. However, if the drug content in the dosing mixture decreases during the period from the time of adjustment of the dosing mixture to the end of the infusion, even the results of large-scale clinical studies will not be reliable.

In the investigation of the necessity of light shielding in the present study, the samples not shielded from light underwent marked changes in external appearance, while the samples shielded from light showed no such changes. However, the drug content decreased over time in the samples not shielded from

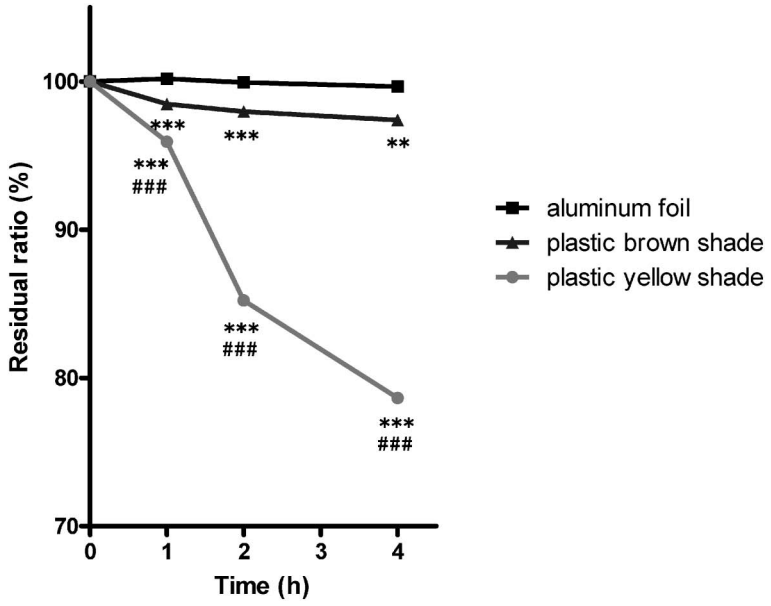

Fig. 3. Percent Residual Carboplatin in the Dosing Solutions (in Three Conditions)

Data are presented as mean±S.E.M. ($n=3$). The statistical significance of the differences between the groups was assessed by Bonferroni's multiple comparison test following one-way ANOVA (**$p<0.01$; ***$p<0.001$ *vs.* aluminum foil, ###$p<0.001$ *vs.* plastic brown shade).

light, as shown in Fig. 2, and formation of degradation products was also suggested, as shown in Fig. 1. A number of degradation products have been reportedly detected in the dosing solution;[1] however, no degradation product was identified in the present study. Pujol *et al.*[1] identified three degradation products of carboplatin in pure water and 5% glucose solution as 1,1-cyclobutanedicarboxilate anion, its protonated forms, and cis-diamminediaquoplatinum (II) complex. Moreover, Lederer and Leipzig-Pagani[2] reported that their electrophoretic separations yielded three cationic and up to five anionic species. In this study we examined under clinical conditions, and our condition was also different from any other previous study. In the exploration for the optimum light-shielding shade in the present study, no changes in external appearance were noted in any of the samples under each set of conditions; however, samples within containers covered by light-shielding shade other than aluminum foil showed a reduction of drug content.

Koubo[12] studied carboplatin solutions in physiological saline and 5% glucose, exposing the solutions to indoor scattered light (0.50±0.01 kLux), and checked for changes in the external appearance, pH, and carboplatin content immediately and at 1, 3, 6, and 24 hours after preparation of the dosing solutions, and found no differences between the light-shielded and non-light-shielded samples. During premarketing development of carboplatin, Oya *et al.*[13] evaluated the stability of carboplatin dissolved in physiological saline and 5% glucose, stored under exposure to indoor scattered light or shielded from light. They reported no reduction in the residual drug content under any of the settings examined. These previous studies were carried out under conditions identical to the setting of light shielding with aluminum foil (0.20±0.12 kLux) in the present study, and the data on the percent residual drug in this study were also similar. However, illuminance under ordinary fluorescent lamps is about 0.2–1.0 kLux, differing considerably from outdoor illuminance (under a roof) value of 48.82±6.01 kLux used in the present study. Torres *et al.*[3] showed that stabilities was defined as <10% loss of initial drug concentration when the samples were exposed under different illumination conditions. Carballar *et al.*[4] demonstrated a degradation of >50% of initial condition under 105, 143, and >200 kLx with a 250 W Xe lamp. The wards and outpatient chemotherapy room at our hospital are designed to have large windows, bearing in mind the psychological state of the patients. For this reason, the illuminance at the window of the wards or the outpatient chemotherapy room during chemotherapy on a fine weather day was as high as about 50 kLux, differing little from outdoor illuminance. Un-

der such settings, reduction in carboplatin content in the dosing solution may be expected to be large. Drawing of the window blinds may suppress reduction in the drug content; however, even then, the drug content may decrease over time.

Regarding differences in light transmission through the various light-shielding shades, the present study revealed a significant difference in the percent residual carboplatin among the different shades employed. This result suggests that the light-shielding shade conventionally used for parenteral hyperalimenation solutions may not be adequate. When aluminum foil with a light-cutting rate of 99.6% was used, percent residual carboplatin was 99.7%, while the percent residual carboplatin was significantly lower (78.6%) when a yellow light-shielding shade (light cutting rate, 78.6%) was used. Thus the type of light-shielding shade used is also important.

The HPLC data suggest the possibility that carboplatin degradation products were formed following exposure to light. We cannot rule out the involvement of these degradation products in the appearance of adverse reactions to carboplatin. To avoid attenuation of drug efficacy and development of adverse reactions due to the formation of degradation products, it would seem advisable to shield the drug solution bottle completely from light.

Such problems as those described above have begun to be unveiled for the first time after pharmacists began to be involved in the clinical management of patients and pay frequent visits to the bedside. We investigated the problem by conducting the present study under experimental conditions close to the clinical setting. Unfortunately, no study of this kind has been carried out yet at the initiative of pharmaceutical companies.

CONCLUSION

Changes in carboplatin-dosing solution during exposure to scattered light, were analyzed and revealed a reduction in the percent residual drug in the dosing solution following such exposure. The results indicate the necessity of completely shielding carboplatin solution from light when it is used for chemotherapy.

If this kind of study is carried out on other drugs from now on, under the settings of indoor scattered light exposure and other clinically prevalent settings, safer and more effective drug therapy can be accomplished.

REFERENCES

1) Pujol M., Girona V., Prat J., Muñoz M., Bolós J., *Int. J. Pharmaceutics*, **146**, 263–269 (1997).
2) Lederer M., Leipzig-Pagani E., *Int. J. Pharmaceutics*, **167**, 223–228 (1998)
3) Torres F., Girona V., Puiol M., Prat and J, de Bolós J, *Int. J. Pharmaceutics*, **129**, 275–277 (1996).
4) Carballar R., Munoz M., Pujol M., Prat J., Girona V., Bolós J., *Biomed. Chromatogr.*, **11**, 119–120 (1997).
5) Zhang Y., Xu Q. A., Trissel L. A., Gilbert D. L., Martinez J. F., *Ann. Pharmacother.*, **31**, 1465–1470 (1997).
6) Prat J., Pujol M., Girona V., Munoz M., Sole L. A., *J. Pharm. Biomed. Anal.*, **12**, 81–84 (1994).
7) Calvert A. H., Newell D. R., Gumbrell L. A., O'Reilly S., Burnell M., Boxall F. E., Siddik Z. H., Judson I. R., Gore M. E., Wiltshaw E., *J. Clin. Oncol.*, **7**, 1748–1756 (1989).
8) Mori T., Hosokawa K., Kinoshita Y., Watanabe A., Yamaguchi T., Kuroboshi H., Kato Y., Yasuda J., Fujita H., Nakata Y., Honjo H., *Int. J. Clin. Oncol.*, **12**, 205–211 (2007).
9) O'Neill V. J., Kaye S. B., Reed N. S., Paul J., Davis J. A., Vasey P. A., *Br. J. Cancer*, **86**, 1385–1390 (2002).
10) Gibbs D. D., Pyle L., Allen M., Vaughan M., Webb A., Johnston S. R., Gore M. E., *Br. J. Cancer*, **86**, 1379–1384 (2002).
11) Yasuda M., Kimura E., Ochiai K., Tada A., Udagawa Y., Aoki D., Nozawa S., Kikuchi Y., Kita T., Nishida M., Tsunoda H., Japanese Journal of Cancer and Chemotherapy, **28**, 493–498 (2001).
12) Koubo B., *J. New Remed. Clin.*, **55**, 581–588 (2006).
13) Oya A., *Antibiotics Chemother.*, **6**, 2186–2191 (1990).