JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2013;43(4)390–395
doi:10.1093/jjco/hyt020
Advance Access Publication 26 February 2013

Renal Toxicity Caused by Brand-name Versus Generic Cisplatin: A Comparative Analysis

Seiji Niho[1,*], Takeharu Yamanaka[2], Shigeki Umemura[1], Shingo Matsumoto[1], Kiyotaka Yoh[1], Koichi Goto[1], Hironobu Ohmatsu[1] and Yuichiro Ohe[1]

[1]Division of Thoracic Oncology, National Cancer Center Hospital East and [2]Section of Translational Medicine and Development, Research Center for Innovative Oncology, National Cancer Center Hospital East, Chiba, Japan

*For reprints and all correspondence: Seiji Niho, Division of Thoracic Oncology, National Cancer Center Hospital East, Kashiwanoha 6-5-1, Kashiwa, Chiba 277-8577, Japan. E-mail: siniho@east.ncc.go.jp

Received December 27, 2012; accepted January 29, 2013

Objective: A generic cisplatin formulation has replaced the brand-name formulation since November 2003 in our hospital. We retrospectively assessed the renal toxicity caused by the brand-name and generic cisplatin formulations.

Methods: The medical records of patients with thoracic malignancy who were treated at our hospital between November 2000 and April 2008 were reviewed. In total, 1296 eligible patients received 80 mg/m^2 of cisplatin: 499 patients were treated with the brand-name cisplatin formulation before November 2003 (Group 1) and 797 patients were treated with the generic formulation after November 2003 (Group 2). We compared the maximum serum creatinine level after chemotherapy in the two groups.

Results: The patient characteristics, including age, sex and performance status, and pretreatment serum creatinine levels were well balanced between the two groups. More patients received four cycles of chemotherapy in Group 2 ($P < 0.0001$). The median (range) of the maximum serum creatinine levels during all the chemotherapy cycles were 1.1 (0.5–4.1) mg/dl and 1.1 (0.5–4.4) mg/dl in Groups 1 and 2, respectively ($P = 0.0237$). The incidence of grade 0 serum creatinine elevations decreased from 47% to 39%, while that of grade 1 serum creatinine elevations increased from 32% to 41% ($P = 0.0094$). The incidence rates of grade 2 or 3 serum creatinine elevations were similar (21 vs. 20%). The time to serum creatinine elevation was also similar in Groups 1 and 2 ($P = 0.161$).

Conclusion: Although grade 1 maximum serum creatinine level was more common in the generic cisplatin formulation group, this was attributed to the larger number of patients receiving four cycles of chemotherapy in this group.

Key words: cisplatin – generic – brand name – renal toxicity

INTRODUCTION

Cisplatin-based chemotherapy is curative for testicular cancer and is active against gynecologic, gastrointestinal, genitourinary, head and neck, and lung cancers as well as other malignant diseases. Carboplatin has the same range of clinical activity as cisplatin but is less nephrotoxic and less emetogenic. Therefore, carboplatin has essentially replaced cisplatin for the treatment of ovarian cancer, lung cancer and a range of other malignancies (1). In some diseases, such as germ cell tumors (2), head and neck cancer (3), and non-small-cell lung cancer (4,5), however, cisplatin is more effective clinically in terms of the response rate and survival.

Cisplatin can cause dose-dependent renal toxicity. Large infusion amounts are needed to prevent cisplatin-induced renal toxicity. Patients are usually prehydrated and posthydrated with at least 2 l of IV fluid to maintain good urine flow. Risk factors for cisplatin nephrotoxicity include the dose and frequency of administration and the cumulative dose of cisplatin, older age, female sex, smoking and hypoalbuminemia (6).

Generic drugs are believed to be bioequivalent to brand-name drugs in terms of dosage form, safety, quality, performance and intended use. They are usually sold at substantial discounts from the branded price. The spread of generic drugs relieves the financial burden of patients' and improves the financial affairs of medical insurance providers (7). Recently, a retrospective analysis from the National Cancer Center Hospital (NCCH) in Tokyo, Japan, demonstrated that renal toxicity was more severe in patients treated with a generic cisplatin formulation than in those treated with the brand-name formulation, especially among male patients (8). To validate these findings, we conducted the same analysis in another patient cohort from the NCCH East, since the same generic cisplatin formulation had been introduced at our hospital, replacing the brand-name formulation, in November 2003.

PATIENTS AND METHODS

Patients were retrospectively selected for this study according to the following criteria, which were identical to those used in the previous analysis (8): (i) a histological or cytological diagnosis of thoracic malignancy; (ii) no prior chemotherapy; (iii) chemotherapy with a regimen that included 80 mg/m^2 of cisplatin; and (iv) receiving treatment as an inpatient at the NCCH East between November 2000 and April 2008. During this period the brand-name cisplatin formulation was administered between November 2000 and October 2003, and CISPLATIN for I.V. infusion (MARUKO), a generic cisplatin formulation, was administered thereafter. Patients with an abnormally elevated serum creatinine (CRN) level prior to the initiation of chemotherapy were excluded from this study. Serum CRN was measured using an enzymatic assay throughout the study period. The upper limit of normal for serum CRN was 1.1 mg/dl for men and 0.9 mg/dl for women.

After 750 ml of intravenous infusion fluids, cisplatin (80 mg/m^2) and 300 ml of fluids were intravenously infused over a 60-min period on day 1 in combination with other chemotherapeutic agents, followed by 40 g of mannitol and 1450 ml of hydration. A total of 2500 ml of hydration fluids, which consisted of 1000 ml of normal saline and 1500 ml of hypotonic crystalloid solution (Solita-T3®), were infused at a rate of 300 ml/h. Twenty milligrams of furosemide was intravenously administered at the end of hydration. One thousand milliliters of intravenous infusion fluids were administered on days 2 and 3 and 500 ml was administered on days 4 and 5 at a rate of 300 ml/h. Antiemetic prophylaxis consisted of a 5HT3 antagonist and 16 mg of dexamethasone on day 1, followed by 8 mg of dexamethasone on days 2 and 3 and 4 mg on days 4 and 5. This sequence of administration was consistently maintained during the study period.

The patients' baseline characteristics including age, sex, performance status (PS), pretreatment CRN level (CRN_{pre}), chemotherapy regimen, number of chemotherapy cycles and maximum CRN level (CRN_{max}) during the first cycle and during all chemotherapy cycles were retrospectively obtained from the patients' medical records. The median CRN_{max} and the Common Toxicity Criteria-Adverse Event (CTC-AE version 3.0) grades of the CRN_{max} were compared in patients treated with the brand-name cisplatin formulation (Group 1) and those treated with the generic formulation (Group 2). The time to serum CRN elevation was defined as the interval between the start of chemotherapy and the development of serum CRN elevation grade 1 or worse. Patients who did not develop serum CRN elevation grade 1 or worse were censored at the end of the cisplatin-based chemotherapy. The time to serum CRN elevation was estimated using the Kaplan–Meier analysis method (9) and was compared between groups using a log-rank test. Mann–Whitney tests were used to evaluate continuous variables and χ^2 tests were used for categorical variables. Multivariate analyses were performed using Cox proportional hazards models to determine the risk factors for the time until serum CRN elevation. Group 1 or 2 and the presence of significant risk factors in the univariate analyses were evaluated using a multivariate analysis. All the reported *P* values were two-sided. GraphPad InStat version 3.10 for Windows (GraphPad Software, San Diego, USA) and PASW Statistics 18 for Windows (SPSS Inc., Chicago, USA) were used for the statistical analyses. The present study was approved by an institutional review board.

RESULTS

Out of 1341 patients assessed for eligibility in this study, 1310 patients met the inclusion criteria; 31 patients were subsequently excluded because of an abnormal CRN_{pre} level. An additional 14 patients were excluded because they were treated with the brand-name cisplatin formulation during the first cycle of chemotherapy but received the generic formulation in subsequent cycles. Therefore, a total of 1296 patients were eligible for this analysis. In total, 499 patients were treated with the brand-name cisplatin formulation (Group 1) and 797 patients were treated with the generic formulation (Group 2) (Fig. 1). The patient characteristics are shown in Table 1. The median age was 63 years (range 27–81 years), and the female patients accounted for 23% of all the patients. No statistical differences in sex, age, PS or CRN_{pre} were observed between the two groups. The most common chemotherapy regimen was cisplatin plus vinorelbine; however, this regimen was less frequently used in Group 2, whereas cisplatin plus gemcitabine was more frequently used. The median number of chemotherapy cycles was three in both groups, but more patients received four cycles of chemotherapy in Group 2 (Fig. 2).

Table 2. Serum creatinine levels and toxicity grades during the first cycle of chemotherapy

	Group 1[a] ($n = 499$), N (%)	Group 2[b] ($n = 797$), N (%)	P value
Median (range)			
Total	0.9 (0.5–4.1)	0.9 (0.4–4.2)	0.1269
Male	1.0 (0.5–4.1)	1.0 (0.6–4.2)	0.0378
Female	0.7 (0.5–1.8)	0.7 (0.4–1.9)	0.3949
CTC-AE grade			
Total			
0	339 (68)	514 (64)	0.6244
1	123 (24)	218 (27)	
2	34 (7)	61 (8)	
3	3 (1)	4 (1)	
Male			
0	282 (72)	418 (68)	0.6732
1	83 (21)	147 (24)	
2	24 (6)	42 (7)	
3	3 (1)	4 (1)	
Female			
0	57 (53)	96 (52)	0.9518
1	40 (38)	71 (38)	
2	10 (9)	19 (10)	

CTC-AE, Common Toxicity Criteria-Adverse Event Ver. 3.0.
[a]Patients treated with the brand-name formulation.
[b]Patients treated with a generic formulation.

Table 3. Serum creatinine levels and toxicity grades during all cycles of chemotherapy

	Group 1[a] ($n = 499$), N (%)	Group 2[b] ($n = 797$), N (%)	P value
Median (range)			
Total	1.1 (0.5–4.1)	1.1 (0.5–4.4)	0.0237
Male	1.2 (0.5–4.1)	1.2 (0.6–4.4)	0.0029
Female	0.8 (0.5–2.6)	0.9 (0.5–2.2)	0.3745
CTC-AE grade			
Total			
0	236 (47)	314 (39)	0.0094
1	159 (32)	326 (41)	
2	100 (20)	150 (19)	
3	4 (1)	7 (1)	
Male			
0	194 (49)	256 (42)	0.0431
1	126 (32)	249 (41)	
2	69 (18)	100 (16)	
3	3 (1)	6 (1)	
Female			
0	42 (39)	58 (31)	0.1455
1	33 (31)	77 (41)	
2	31 (29)	50 (27)	
3	1 (1)	7 (1)	

[a]Patients treated with the brand-name cisplatin formulation.
[b]Patients treated with a generic cisplatin formulation.

factor for the time to serum CRN elevation (HR: 1.096, 95% CI: 0.943–1.276) (Table 5).

DISCUSSION

A previous retrospective analysis from the NCCH in Tokyo, Japan, demonstrated that a grade 2 or 3 CRN_{max} was observed in 9.4% of the male patients treated with the brand-name cisplatin formulation and 20.9% of the male patients treated with a generic formulation identical to that used in our study during all the chemotherapy cycles ($P < 0.001$) (8). In our study, grade 2 or 3 CRN_{max} was observed in 19 and 17% of the male patients of the two groups, respectively. Three thousand milliliters on day 1 and 2000 ml of intravenous infusion fluids on days 2–5 were administered at the NCCH, with identical antiemetic prophylaxis of a 5HT3 antagonist and dexamethasone and 40 g of mannitol on day 1. However, 2,500 ml of intravenous infusion fluids on day 1, 1000 ml on days 2 and 3, and 500 ml on days 4 and 5 were administered in our hospital. The median age of patients was 60 years in the NCCH study and 63 years in this study. The reason why our study could not confirm a

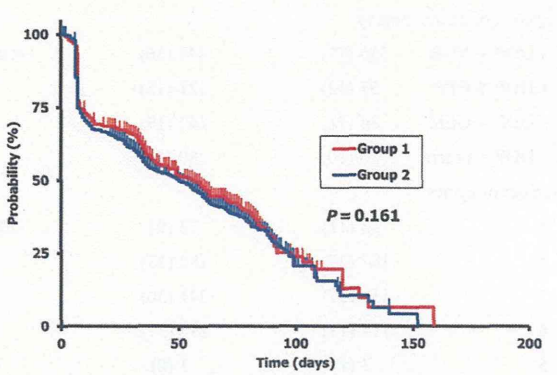

Figure 3. Kaplan–Meier curves for time to serum creatinine elevation. The patients in Group 1 were treated with the brand-name cisplatin formulation, while the patients in Group 2 were treated with the generic formulation. The probability means the percentage of patients who did not develop elevation of the serum creatinine level. Patients who did not develop serum creatinine elevation grade 1 or worse were censored at the end of the cisplatin-based chemotherapy. Therefore, these Kaplan–Meier curves reveal when serum creatinine elevated after the initiation of cisplatin-based chemotherapy.

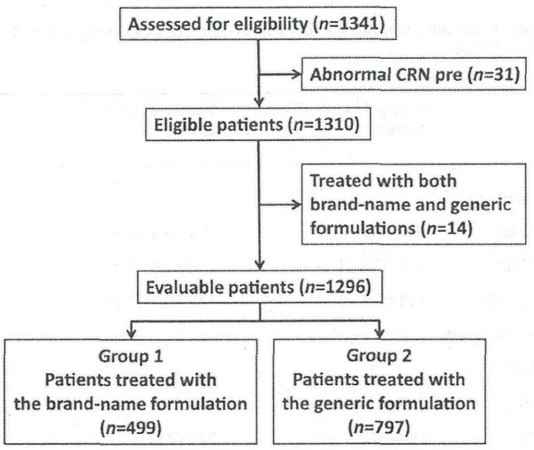

Figure 1. Diagram of the study. CRN_{pre}, pretreatment serum creatinine level.

Table 1. Patient characteristics

	Group 1[a] ($n = 499$), N (%)	Group 2[b] ($n = 797$), N (%)	P value
Sex			
Male	392 (79)	611 (77)	0.4532
Female	107 (21)	186 (23)	
Age (years)			
Median (range)	62 (28–78)	63 (27–81)	0.7368
Performance status			
0–1	486 (97)	788 (99)	0.0744
2–3	13 (3)	9 (1)	
Pretreatment serum creatinine level			
Median (range)	0.7 (0.4–1.1)	0.7 (0.4–1.1)	0.1742
Regimen of chemotherapy			
CDDP + VNR	356 (71)	447 (56)	<0.0001
CDDP + ETP	57 (12)	121 (15)	
CDDP + GEM	36 (7)	149 (19)	
CDDP + others	50 (10)	80 (10)	
Number of cycles			
1	53 (11)	72 (9)	<0.0001
2	187 (37)	184 (23)	
3	136 (27)	241 (30)	
4	114 (23)	294 (37)	
5	3 (1)	1 (0)	
6	6 (1)	5 (1)	
Median (range)	3 (1–6)	3 (1–6)	<0.0001

CDDP, cisplatin; VNR, vinorelbine; GEM, gemcitabine; others included irinotecan, docetaxel, vinorelbine + mitomycin C, paclitaxel and S-1.
[a]Patients treated with the brand-name cisplatin formulation.
[b]Patients treated with a generic cisplatin formulation

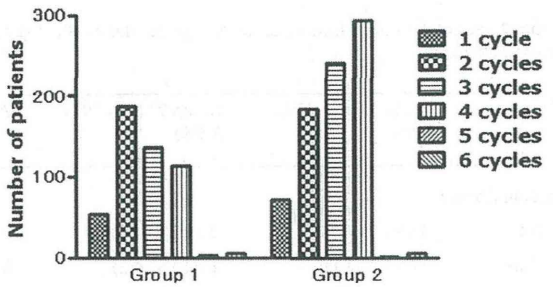

Figure 2. Number of chemotherapy cycles in 499 patients treated with the brand-name cisplatin formulation (Group 1) and 797 patients treated with the generic formulation (Group 2).

The median (range) CRN_{max} levels during the first cycle of chemotherapy were 1.0 (0.5–4.1) mg/dl and 1.0 (0.6–4.2) mg/dl in the male patients in Groups 1 and 2, respectively ($P = 0.0378$), whereas they were 0.7 (0.5–1.8) mg/dl and 0.7 (0.4–1.9) mg/dl in the female patients in Groups 1 and 2, respectively ($P = 0.3949$). The CTC-AE grade for CRN_{max} during the first cycle was not statistically different between Groups 1 and 2 in both male ($P = 0.6732$) and female patients ($P = 0.9518$) (Table 2).

The median (range) CRN_{max} levels during all the chemotherapy cycles were 1.1 (0.5–4.1) mg/dl and 1.1 (0.5–4.4) mg/dl in all the patients in Groups 1 and 2, respectively ($P = 0.0237$). The median (range) CRN_{max} levels during all the cycles of chemotherapy were 1.2 (0.5–4.1) mg/dl and 1.2 (0.6–4.4) mg/dl in the male patients in Groups 1 and 2, respectively ($P = 0.0029$), whereas they were 0.8 (0.5–2.6) mg/dl and 0.9 (0.5–2.2) mg/dl in the female patients in Groups 1 and 2, respectively ($P = 0.3745$). The CTC-AE grade for CRN_{max} during all the cycles was statistically different between Groups 1 and 2 in the male patients ($P = 0.0431$). Grade 0 CRN_{max} decreased from 49% to 42%, whereas grade 1 CRN_{max} increased from 32% to 41% between the male patients in Groups 1 and 2. An identical tendency was observed in the female patients. Grade 0 CRN_{max} decreased from 39% to 31%, whereas grade 1 CRN_{max} increased from 31% to 41% between the female patients in Groups 1 and 2 ($P = 0.1455$). In all the patients, grade 0 CRN_{max} decreased from 47% to 39% and grade 1 CRN_{max} increased from 32% to 41% between Groups 1 and 2 ($P = 0.0094$). Grade 2 or 3 CRN_{max} was not different between Groups 1 and 2 in both the male and female patients (Table 3). The time to serum CRN elevation was not statistically different between Groups 1 and 2 ($P = 0.161$) (Fig. 3). A male sex or an age of 71 years or older was significantly associated with a shorter time to a serum CRN elevation grade 1 or worse in a univariate analysis (Table 4).

A multivariate analysis showed that a female sex [hazard ratio (HR): 1.528, 95% confidence interval (CI): 1.296–1.803] and an age of 71 years or older (HR: 1.362, 95% CI: 1.127–1.645) were significant risk factors for the time to serum CRN elevation. Group 2 was not a significant risk

Table 4. Time to serum creatinine elevation grade 1 or worse (univariate analysis)

	Median time to serum creatinine elevation (days)	95% confidence interval	*P* value
Gender			
Male	60	53.7–66.3	<0.001
Female	29	21.5–36.5	
Age			
≤70 years old	56	49.9–62.1	0.003
≥71 years old	34	19.4–48.6	
Cisplatin group			
Group 1	56	46.1–65.9	0.161
Group 2	50	42.0–58.0	

Patients in Group 1 were treated with the brand-name cisplatin formulation, whereas patients in Group 2 were treated with a generic cisplatin formulation.

Table 5. Multivariate analysis of risk factors associated with time to serum creatinine elevation.

Variable	Hazard ratio	95% confidence interval	*P*-value
Female sex	1.528	1.296–1.803	<0.001
Age ≥71 years old	1.362	1.127–1.645	0.001
Group 2	1.096	0.943–1.276	0.229

Patients in Group 2 were treated with a generic cisplatin formulation.

high frequency of grade 2–3 CRN_{max} in the generic cisplatin formulation group is unknown. Although grade 1 CRN_{max} was more common in the generic cisplatin formulation group, this was attributed to the larger number of patients receiving four cycles of chemotherapy in this group. A multivariate analysis also demonstrated that the generic cisplatin formulation group was not a statistically significant risk factor associated with the time to serum CRN elevation. We concluded that the generic cisplatin formulation did not increase renal toxicity compared with the brand-name cisplatin formulation.

The main objective of using generic drugs, rather than the brand-name drugs, is cost savings (10). Generic drugs are usually approved without clinical trials, although the same high quality, strength, purity and stability as brand-name drugs are required. Our study suggested that the generic cisplatin formulation did not increase renal toxicity, compared with the brand-name formulation. This kind of survey is needed for other generic drugs, especially anticancer drugs that can cause severe or life-threatening toxicities. We believe it is important to confirm the safety of generic drugs. If possible, it is desirable to conduct clinical trials to evaluate the safety and efficacy of generic drugs before approval. However, a large-scale clinical trial needs great cost and is impracticable to conduct.

Magnesium was not included in the hydration fluid. Several randomized trials have demonstrated that the addition of magnesium is effective for reducing cisplatin-induced renal toxicity (11,12). Grade 2–3 CRN_{max} was observed in ~20% of patients, which sounds still high. A four-arm cooperative study in Japan demonstrated that the incidence of grade 2–3 serum CRN elevation was 7–9% in the cisplatin-based chemotherapy group (13). We analyzed consecutive patients who were treated with cisplatin-based chemotherapy; therefore, more patients who had co-morbidity and were ineligible for clinical trials might have been included in this study, resulting in a higher incidence of grade 2–3 serum CRN elevation than those in clinical trials. To reduce cisplatin-induced renal toxicity, we have added magnesium to the hydration fluid administered prior to cisplatin since 2010. We plan to analyze whether preloading with magnesium before chemotherapy can further reduce cisplatin-induced renal toxicity.

Our retrospective analysis has several limitations. First, other risk factors for cisplatin nephrotoxicity, such as smoking status, pretreatment serum albumin level or the co-administration of non-steroidal anti-inflammatory agents, were not investigated. Secondly, aprepitant, which is a standard antiemetic agent nowadays (14), was approved in late 2009 in Japan. None of the patients in our study received aprepitant. The introduction of aprepitant might reduce anorexia induced by cisplatin and might prevent dehydration and renal dysfunction. Thirdly, the frequency of chemotherapy delay, dose reduction or termination of chemotherapy due to renal toxicity was not investigated. This information will be helpful to understand the clinical impact of the renal toxicity.

In conclusion, the incidence of grade 2–3 CRN_{max} was not higher in the generic cisplatin group, although the incidence of grade 1 CRN_{max} was higher. However, more patients in the generic cisplatin formulation group received four cycles of chemotherapy than in the brand-name cisplatin group. The time to serum CRN elevation was not statistically different between the two groups.

Acknowledgements

The authors thank Eriko Imai for her assistance in the preparation of this manuscript.

Funding

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare, Japan.

Conflict of interest statement

None declared.

References

1. Reed E. Cisplatin and its analogs. In: DeVita VT, Lawrence TS, Rosenberg SA, editors. *Cancer: Principles & Practice of Oncology*. 8th edn. Philadelphia: Wolters Kluwer Lippincott Williams & Wilkins 2008;419–26.
2. Horwich A, Sleijfer DT, Fossa SD, et al. Randomized trial of bleomycin, etoposide, and cisplatin compared with bleomycin, etoposide, and carboplatin in good-prognosis metastatic nonseminomatous germ cell cancer: a Multiinstitutional Medical Research Council/European Organization for Research and Treatment of Cancer Trial. *J Clin Oncol* 1997;15:1844–52.
3. De Andres L, Brunet J, Lopez-Pousa A, et al. Randomized trial of neoadjuvant cisplatin and fluorouracil versus carboplatin and fluorouracil in patients with stage IV-M0 head and neck cancer. *J Clin Oncol* 1995;13:1493–500.
4. Ardizzoni A, Boni L, Tiseo M, et al. Cisplatin- versus carboplatin-based chemotherapy in first-line treatment of advanced non-small-cell lung cancer: an individual patient data meta-analysis. *J Natl Cancer Inst* 2007;99:847–57.
5. Hotta K, Matsuo K, Ueoka H, et al. Meta-analysis of randomized clinical trials comparing cisplatin to carboplatin in patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2004;22:3852–9.
6. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of cisplatin nephrotoxicity. *Toxins (Basel)* 2010;2:2490–518.
7. Cornes P. The economic pressures for biosimilar drug use in cancer medicine. *Target Oncol* 2012;7(Suppl. 1):57–67.
8. Sekine I, Kubota K, Tamura Y, et al. Innovator and generic cisplatin formulations: comparison of renal toxicity. *Cancer Sci* 2011;102:162–5.
9. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457–81.
10. Haas JS, Phillips KA, Gerstenberger EP, Seger AC. Potential savings from substituting generic drugs for brand-name drugs: medical expenditure panel survey, 1997–2000. *Ann Intern Med* 2005;142:891–7.
11. Bodnar L, Wcislo G, Gasowska-Bodnar A, et al. Renal protection with magnesium subcarbonate and magnesium sulphate in patients with epithelial ovarian cancer after cisplatin and paclitaxel chemotherapy: a randomised phase II study. *Eur J Cancer* 2008;44:2608–14.
12. Willox JC, McAllister EJ, Sangster G, Kaye SB. Effects of magnesium supplementation in testicular cancer patients receiving cis-platin: a randomised trial. *Br J Cancer* 1986;54:19–23.
13. Ohe Y, Ohashi Y, Kubota K, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. *Ann Oncol* 2007;18:317–23.
14. Basch E, Prestrud AA, Hesketh PJ, et al. Antiemetics: American Society of Clinical Oncology clinical practice guideline update. *J Clin Oncol* 2011;29:4189–98.

Ann Surg Oncol (2013) 20:798–803
DOI 10.1245/s10434-012-2567-0

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – GASTROINTESTINAL ONCOLOGY

Outcome and Status of Microsatellite Stability in Japanese Atomic Bomb Survivors with Early Gastric Carcinoma

Manabu Yamamoto, MD, PhD, FACS[1,2], Kenichi Taguchi, MD, PhD[3], Takeharu Yamanaka, PhD[4], Ayumi Matsuyama, MD, PhD[1], Keiji Yoshinaga, MD, PhD[1], Shinichi Tsutsui, MD, PhD[1], and Teruyoshi Ishida, MD, PhD[1]

[1]Department of Surgery, Hiroshima Atomic Bomb Survivors Hospital, Hiroshima, Japan; [2]Department of Gastroenterological Surgery, National Kyushu Cancer Center, Fukuoka, Japan; [3]Department of Pathology, National Kyushu Cancer Center, Fukuoka, Japan; [4]Research Center for Innovation Oncology, National Cancer Center Hospital East, Chiba, Japan

ABSTRACT

Background. In the decade after the 1945 atomic bombing of Hiroshima, a high incidence of leukemia was observed among atomic bomb survivors. However, the incidence of other cancers gradually increased, while that of leukemia decreased after this period. We evaluated the clinical outcome of early gastric cancer and microsatellite stability over a long-term period in atomic bomb survivors.

Methods. The results of surgical treatment for early gastric cancer were reviewed for 117 atomic bomb survivors and 394 control patients between 1995 and 2006. In addition, immunohistochemical staining for hMSH2 and hMLH1 expression was performed to evaluate the status of microsatellite stability in 57 atomic bomb survivors and 82 control patients.

Results. The long-term survival rate for early gastric cancer in atomic bomb survivors was significantly lower than that in control patients ($p < 0.01$). Multivariable analysis revealed that age and sex were significant and independent prognostic factors for early gastric cancer. Defective hMSH2 and/or hMLH1 expression was also significantly higher in survivors than in control patients ($p < 0.001$). Logistic regression analysis revealed that atomic bomb survivorship was related to defective hMSH2 and/or hMLH1 expression.

Conclusions. The prognosis of early gastric cancer in atomic bomb survivors was poor and was related to age and sex, rather than to being an atomic bomb survivor. Furthermore, a higher rate of defective hMSH2 and/or hMLH1 expression was observed in the survivors.

First Received: 13 January 2012;
Published Online: 13 November 2012

M. Yamamoto, MD, PhD, FACS
e-mail: yamamoto.m@nk-cc.go.jp

In the decade after the 1945 atomic bombing of Hiroshima and Nagasaki, a high incidence of leukemia was observed among atomic bomb survivors.[1] However, the incidence of other cancers, including gastric cancer, gradually increased after this period, whereas that of leukemia decreased.[2] The carcinogenic effect of ionizing radiation on the stomach has been confirmed in animal models.[3] Although a dose-dependent carcinogenic effect was demonstrated in atomic bomb survivors with leukemia in 1982, the same effect in the stomach and lung remains unidentified.[2] To date, few clinical reports have evaluated the clinicopathological features, surgical treatment, and long-term course of carcinoma in these survivors. In particular, Suehiro et al. [4] reported that the prognosis of gastric cancer in atomic bomb survivors was similar to that in control patients in the short term.

In general, the frequency of multiple primary cancers has been reported to be 10 % in Japan.[5,6] In particular, the frequency of synchronous or metachronous cancers of the colorectum and lung is reportedly higher than that of other cancers in Japan.[5] According to previous studies, the prognosis of early gastric cancer patients was usually influenced by the site of multiple primary cancers.[6] However, the biological nature and prognosis of multiple primary cancers were not classified. We have reported the relationship between double primary cancers (stomach and colorectum) and defective hMSH2 and/or hMLH1 expression.[7]

This study aimed to review the results of surgical treatment for early gastric cancer in 117 atomic bomb

survivors and 394 control patients, and to compare the clinicopathological characteristics and immunohistochemical staining of hMSH2 and hMLH1 expression between these two groups.

PATIENTS AND METHODS

Patients

This study included 511 patients with early gastric cancer who underwent surgery at the Department of Surgery, Hiroshima Atomic Bomb Survivors Hospital, Hiroshima, Japan. Several clinicopathological factors, including sex, mean age at the time of surgery, surgical method, histology, depth of tumor invasion, lymph node metastasis, lymphatic and venous invasion, and occurrence of second primary cancer, were evaluated as potential prognostic factors. Surgical and clinicopathological evaluations were performed according to the tumor, node, metastasis staging system classification of gastric cancer.[8] Second primary carcinomas were determined by reviewing the patients' medical records, including medical history, laboratory data, and the results of ultrasonography, computed tomography, and endoscopy.

The diagnostic criteria of second primary cancer was based on those listed by Warren and Gates: (1) presence of cancer, (2) presence of cancer in separate locations, and (3) verification that one cancer was not a metastatic lesion of another cancer.[9]

Immunohistochemical Study

Immunohistochemical analyses for hMSH2 and hMLH1 expression were performed on 4-mm-thick, formalin-fixed, paraffin-embedded tissue sections of colorectal and gastric cancers using the Dako EnVision + System, HRP (horseradish peroxidase) (DAB) (DakoCytomation California, Carpinteria, CA). The sections were deparaffinized in xylene and dehydrated through graded alcohol to water. The sections were then immersed in Dako target retrieval solution with a high pH (10.0), subjected to heart-induced antigen retrieval in a water bath at 98 °C for 40 min, and cooled slowly at room temperature for 20 min. The endogenous peroxidase activity was blocked by incubation with blocking solution (0.03 % H_2O_2 and sodium azide), and the specimens were washed in Tris-buffered saline (TBS; 50 mmol/l Tris-HCl, 150 mmol/l NaCl, pH 7.6). The sections were incubated overnight at 4 °C with one of the following mouse monoclonal antibodies: clone FE11 (antibody against MSH2; 1:100; Oncogene Research Products, La Jolla, CA) or clone G168–15 (antibody against MLH1; 1:100; BD Biosciences, San Jose, CA). Next, they were diluted with Dako antibody diluent with background reducing components. After rinsing three times with TBS, the sections were incubated with polymer solution (HRP-labeled dextran polymer conjugated to goat polyclonal anti-mouse immunoglobulin antibody) for 60 min at room temperature. The sections were washed three times with TBS, and finally, they were incubated with diaminobenzidine and H_2O_2, counterstained in hematoxylin, washed in tap water, dehydrated with graded alcohol, cleared in xylene, and mounted on slides. For the negative controls, the Dako antibody diluent was replaced by background reducing components without the primary antibodies for each immunostain.[7]

Immunohistochemical analyses for hMSH2 and hMLH1 expression were performed with the stomach mucosa specimens of patients who underwent gastrectomy for benign disease. Normal mucosa was used as a positive control.

The hMSH2 and hMLH1 expression was evaluated independently by two observers. A total of 1,000 tumor cells in five representative high-power fields (200 tumor cells for each field) were counted under a photomicroscope. The score from each individual case was calculated as the average of the results recorded by the two observers. The overall extent of immunoreaction was evaluated using the following criteria for hMSH2 and hMLH1 expression: 0–5 % staining was considered as negative staining, while anything >5 % was regarded as positive staining.

Follow-up

After discharge, all patients were followed up with physical and blood examinations every 4–12 weeks; in addition, chest X-rays, computed tomography, and ultrasonography of the abdomen were performed every 3–6 months for the first year. Thereafter, follow-ups were conducted at intervals ranging from 6–12 months. In some cases, other cancers were determined by endoscopy, biopsy, or surgery. The median follow-up period for patients in this study was 50 months (range 3–143 months).

Statistical Analysis

All data are shown as the prevalence or mean (±SD). Clinicopathological factors other than age were compared by the χ^2 test. Age was compared by Student's t test. The survival rate was analyzed using the Kaplan–Meier method, and the log rank test was used for comparisons of survival rates. A p value of <0.05 was considered statistically significant. In addition, prognostic factors associated with decreasing survival rates were determined by Cox regression analysis.[10] The proportional hazard assumption was assessed graphically. As the predictor of defective hMSH2 and/or hMLH1 expression, a logistic regression

analysis was performed. All statistical analysis was used the JMP software (SAS, Cary, NC).

RESULTS

Clinicopathological Characteristics of Patients

The clinicopathological characteristics of the atomic bomb survivors and control patients are shown in Table 1. Age, histology, and occurrence of second primary cancer were significantly different between the two groups. Older age, well-differentiated type, and occurrence of second primary cancer predominated in the survivors compared with control patients.

TABLE 1 Clinicopathological features in patients with early gastric cancers

Feature	Characteristic	Control patients ($n = 394$)	Atomic bomb survivors ($n = 117$)	p
Sex	Male	287	79	0.262
	Female	107	38	
Age (years)		61.7 ± 11.4	70.8 ± 7.6	0.001
Gastric resection	Distal	294	81	
	Total	66	19	0.174
	Local	29	15	
	Others	5	2	
Histology	Well differentiated	262	101	0.001
	Poorly differentiated	132	16	
Depth of tumor invasion	m	229	64	0.511
	sm	165	53	
Lymph node metastasis	Positive	37	12	0.780
	Negative	357	105	
Lymphatic involvement	Positive	107	38	0.259
	Negative	284	78	
	Unclear	3	1	
Vessel involvement	Positive	24	10	
	Negative	367	106	0.348
	Unclear	3	1	
Second primary cancer	Positive	27	20	0.001
	Negative	367	97	

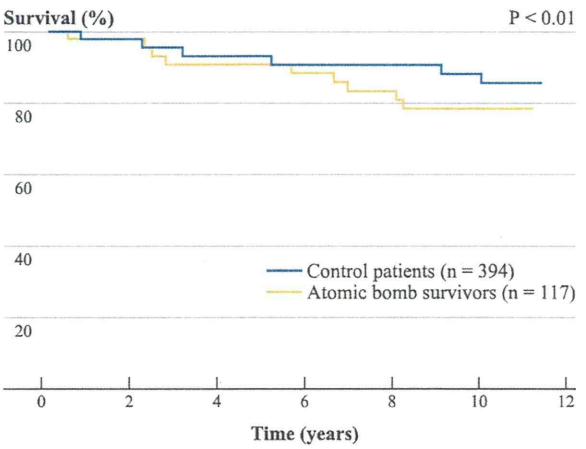

FIG. 1 Ten-year survival rates with early gastric cancer in the control patients and atomic bomb survivors groups

Long-term Survival Rate and Multivariate Analysis

Figure 1 illustrates the long-term survival curves of all the patients. The 5-year survival rate was 91 and 92 %, and the 10-year survival rate was 77 and 87 % in survivors and control patients, respectively. Long-term survival was significantly different between the two groups ($p < 0.01$).

Age, sex, histology, tumor invasion, lymph node metastases, the occurrence of second primary cancer, and atomic bomb survivorship for overall survival were considered independent prognostic factors. Age, sex, histology, and atomic bomb survivorship were significant and independent prognostic factors in the univariate analysis. According to the multivariate analysis, age and sex were significant and independent prognostic factors, with a relative risk of 1.7 and 1.6, respectively (Table 2).

Immunohistochemistry for hMLH1 and hMSH2 Expression

We performed an immunohistochemical analysis to evaluate hMSH2 and hMLH1 expression in 57 control patients and 82 atomic bomb survivors; these subjects were adjusted for the same characteristic findings as those of the 117 control patients and 394 atomic bomb survivors.

Figure 2 shows the results of immunohistochemical staining for hMSH2 and hMLH1 expression in the normal and gastric cancer tissues of both control patients and atomic bomb survivors. hMSH2 and hMLH1 expression was maintained in the cancer tissues of most control patients. The rate of defective hMSH2 and/or hMLH1 expression in atomic bomb survivors was significantly higher than that in control patients ($p < 0.001$, Table 3). However, the survival rates between patients with negative

TABLE 2 Univariate and multivariate analysis of survival in 511 patients

Variable	Univariate analysis			Multivariate analysis		
	p	Hazard ratio	95 % CI	*p*	Hazard ratio	95 % CI
Age	0.0003	1.7300	1.2946–2.3150	0.0015	1.6591	1.2156–2.2718
Sex	0.0381	1.4778	1.0201–2.3103	0.0113	1.6415	1.1112–2.6015
Histology	0.0382	1.4513	1.0192–2.2043	0.5064	1.1462	0.5602–1.2872
Tumor invasion	0.1855	1.2139	0.9013–1.6208	0.4844	1.1130	0.8214–1.5051
Lymph node metastasis	0.4671	1.1803	0.7283–1.7439	0.3340	1.2677	0.7633–1.9469
Second primary cancer	0.2724	1.2520	0.8232–1.7859	0.4688	1.1590	0.7589–1.6629
Atomic bomb survivor	0.0194	1.4489	1.0645–1.9400	0.1069	1.2996	0.9431–1.7934

CI confidence interval

and positive hMSH2 and/or hMLH1 expression were not significantly different in both groups (Fig. 3).

Logistic Regression Analysis

According to multivariate logistic regression analysis, atomic bomb survivorship was found to be significantly and independently related to defective hMSH2 and/or hMLH1 expression, with an odds ratio of 3.4 (Table 4).

DISCUSSION

Various differences between atomic bomb survivors and control patients have been previously reported between 1970 and 1980, with significant differences in age, sex, and histology distributions.[4] Similarly, in a study conducted between 1995 and 2006, significant differences were observed in age, histology, and occurrence of second primary cancer between the survivors and control patients with early gastric cancer. A higher rate of elderly patients, well-differentiated adenocarcinoma, and occurrence of second primary cancer after early gastric cancer was observed in atomic bomb survivors than in control patients. We previously reported that the prognosis in atomic bomb survivors was worse than that in control patients.[11] The 10-year survival rate for atomic bomb survivors with early gastric cancer was significantly lower than that in control patients (Fig. 1). Age and sex were independent factors for overall survival according to the multivariate analysis, though age, sex, histology, and being an atomic bomb survivor were independent factors for overall survival according to the univariate analysis. Histology and atomic bomb survivorship were dependent on age.

It is generally accepted that ionizing radiation damages cellular DNA and causes mutations.[12] Kamada et al. [13] in a 1976 study, found chromosomal aberrations in 20–40 % of the T-lymphocytes and bone marrow cells of atomic bomb survivors exposed to radiation near the hypocenter. An

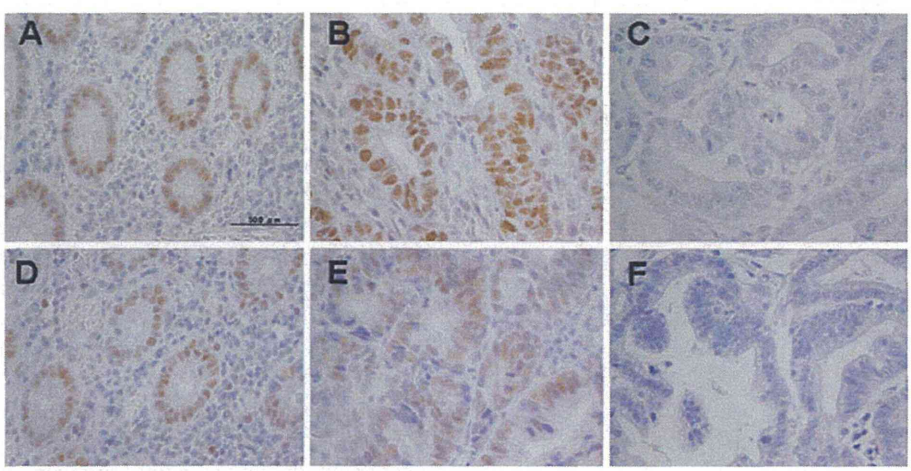

FIG. 2 Immunohistochemical staining for hMSH2 and hMLH1 expression. Sections were stained using antibodies for either hMSH2 (**a–c**) or hMLH1 (**d–f**). **a**, **d** Normal tissue specimens. **b**, **e** Tumor tissue specimens of a control patient. **c**, **f** Tumor tissue specimens of an atomic bomb survivor