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Risk factors contributing to urinary protein expression resulting from bevacizumab combination chemotherapy

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Proteinuria following administration of bevacizumab is reported to be a specific adverse effect, but the risk factors for proteinuria have not been elucidated. In this study, the risk factors for urinary protein expression resulting from bevacizumab combination chemotherapy were investigated. The subjects were 47 patients aged ≥ 20 years who had received bevacizumab combination chemotherapy at Gifu Municipal Hospital between February 2010 and February 2011. A total of 13 patients were excluded based on exclusion criteria; of the remaining 34 patients, 24 (70.6%) were assigned to the urinary protein non-expression group, and 10 (29.4%) were assigned to the urinary protein expression group. The results of multivariate logistic regression analysis revealed a significant difference in systolic blood pressure (≥ 130 mmHg) between the two groups (OR: 14.499, 95%CI: 1.326−158.577, p = 0.028). This finding shows that systolic blood pressure (≥ 130 mmHg) is a risk factor for urinary protein expression resulting from bevacizumab combination chemotherapy.

1. Introduction

The molecularly targeted drug bevacizumab (BV) is a recombinant humanized monoclonal antibody for human vascular endothelial growth factor (VEGF). In tumor cells, BV binds specifically to VEGF and interferes with its biological activity. As a result, it inhibits angiogenesis in tumor tissue and displays an antitumor effect (Presta et al. 1997). BV has been shown to be effective when used in combination with standard chemotherapy for advanced or recurrent colorectal cancer in which curative resection is not possible, for unresectable advanced or recurrent non-small-cell lung cancer with the exception of squamous cell carcinoma, and for inoperable or recurrent breast cancer (Saltz et al. 2008; Fuchs et al. 2007; Reck et al. 2009). Because the action mechanism of molecularly targeted drugs differs from that of conventional cytotoxic anticancer agents, attention must be paid to specific adverse effects. Typical adverse effects of BV include elevated blood pressure, proteinuria, and bleeding. In particular, the incidence of proteinuria is reported to be 30–40%, the majority of which is grade 1 or 2, in rare cases reaching grade 3 or 4 (Iwasa and Kato 2008). Drug discontinuation is considered when proteinuria exceeds grade 3 or when it reaches grade 4 (Gordon and Cunningham 2005), and in cases when BV is discontinued, its antitumor effect is insufficient. When BV and chemotherapy are combined, it is reported that the risk of proteinuria is 4.79 times higher than with chemotherapy alone (Wu et al. 2010). However, there are few reports on the development of proteinuria following administration of BV, and the risk factors have not been elucidated.

In this study, a retrospective survey of patients who received BV combination chemotherapy was conducted, and the risk factors that contribute to the development of proteinuria were investigated.

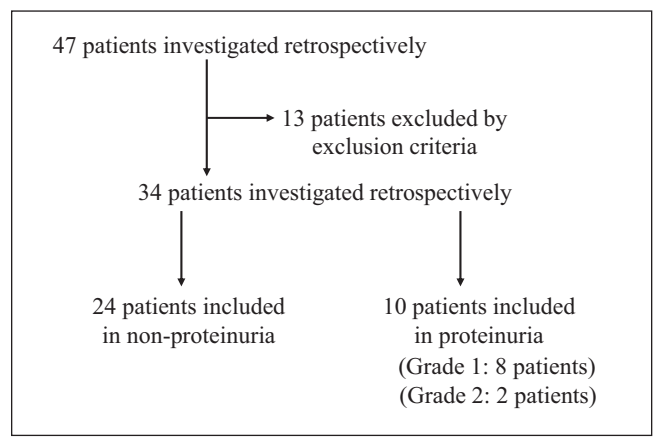

Fig. 1: Subject selection and number of subjects analyzed

2. Investigations and results

2.1. Patient background

Of the 47 patients who underwent BV combination therapy, 4 patients for whom urine tests were not done and 9 in whom proteinuria was present at the time of the first urine test were excluded (Fig. 1). Of the 34 patients in whom proteinuria onset could be followed, 24 (70.6%) did not develop proteinuria (non-proteinuria group), and 10 (29.4%) did develop proteinuria (proteinuria group). Among patients in whom proteinuria occurred, the proteinuria was grade 1 in 8 patients (23.5 %) and grade 2 in 2 patients (5.9 %). Among the 34 patients, 19 were men and 15 were women, with a mean age of 63.9 ± 11.9 (mean ± SD) years. The type of carcinoma was rectal cancer in

Table 1: Demographic characteristics of the patients

		n	Mean ± SD (Median)
Total patients		34	
Gender (male/female)		19/15	
Age (years)		34	63.9 ± 11.9 (65)
BMI		33	21.9 ± 3.3 (21.4)
Diagnosis	Rectal cancer	12	
	Colon cancer	10	
	Non-small-cell lung cancer	12	
Multiagent regimen	mFOLFOX [a)]	11	
	XEROX [b)]	8	
	PMT [c)]	8	
	FOLFIRI [d)]	2	
	CBDCA + TAX [e)]	2	
	CDDP + PMT [f)]	1	
	Erlotinib [g)]	1	
	S-1/L-OHP(SOX) [h)]	1	
Stage	III	8	
	IV	26	

a) fluorouracil, leucovorin, oxaliplatin, b) capecitabine, oxaliplatin, c) pemetrexed, d) fluorouracil, leucovorin, irinotecan, e) carboplatin, paclitaxel, f) cisplatin, pemetrexed, g) erlotinib, h) tegafur gimeracil oteracil potassium, oxaliplatin

12 patients, colon cancer in 10, and non-small-cell lung cancer in 12. The combination regimen was mFOLFOX in 11 patients, XEROX and PMT in 8, FOLFIRI and CBDCA + TAX in 2, and CDDP + PMT, erlotinib, and S-1/L-OHP in 1. Other patient information is shown in Table 1.

2.2. Investigation of risk factors affecting proteinuria onset

Univariate analysis of risk factors contributing to the onset of proteinuria showed the two items of dose per body weight and fasting blood sugar level to be factors contributing to expression of urinary protein (Table 2).

Factors with $p < 0.25$ were sex, colon cancer, dose per body weight, whether or not antihypertensives were administered, combination regimen (mFOLFOX, PMT, CBDCA + TAX), systolic blood pressure ($\geq$ 130 mmHg), and fasting blood sugar level.

With onset of proteinuria as the response variable, a multivariate logistic regression analysis was conducted with sex, colon cancer, dose per body weight, whether or not antihypertensives were administered, and systolic blood pressure, which were factors for which $p < 0.25$ on univariate analysis, as explanatory variables. The cutoff value for the continuous variable in dose per body weight, obtained from the ROC curve, was 5.0 mg/kg. This cutoff value was used in the multivariate logistic regression analysis. In consideration of the problems of blood sugar levels with missing values and multicollinearity, the combination regimens mFOLFOX, PMT, CBDCA + TAX were not included as an explanatory variable. The results are shown as a forest plot in Fig. 2. Odds ratios (OR) and 95% confidence intervals (95%CI) are shown on the right side of the figure. The results showed a significant difference for systolic blood pressure ($\geq$ 130 mmHg) (OR: 14.499, 95%CI: 1.326–158.577, p=0.028).

3. Discussion

Proteinuria is reported to be a specific adverse effect of BV, and BV is discontinued if proteinuria reaches grade 3 (4 + or > 3.5 g/24 h) or grade 4 (nephrotic syndrome) (Gordon and Cunningham 2005). In such cases, the antitumor effect of BV is insufficient. To obtain an antitumor effect from BV, healthcare workers need to be aware of the risk factors for proteinuria and monitor patients receiving BV. However, the risk factors affecting the onset of proteinuria following administration of BV have not been identified. Therefore, the present study was performed to investigate the risk factors that affect the onset of proteinuria in BV combination chemotherapy.

The percentage of patients with proteinuria in this study was 29.4% (10/34). All of these patients had grade 1 or grade 2 proteinuria; none had grade $\geq$ 3. Iwasa and Kato (2008) reported incidences of proteinuria of 30% to 40% (grade unknown), corresponding with the results of the present study. In addition, Wu et al. (2010). reported an incidence of 2.2% for severe proteinuria of grade $\geq$ 3, but patients in the present survey had only grade 1 or grade 2 proteinuria.

In the present study, systolic blood pressure ($\geq$ 130 mmHg) was shown to be an independent risk factor for the development of proteinuria in BV combination chemotherapy. Blood pressure is reported to be related to proteinuria (Zhu et al. 2007), and when systolic blood pressure at the start of BV combination chemotherapy is $\geq$ 130 mmHg, measures such as administration of an antihypertensive agent are necessary. The fact that antihypertensive agents were not identified as a risk factor in the present study also indicates that administration of antihypertensive agents for appropriate blood pressure management may reduce the risk of proteinuria and help prevent increases in the severity of proteinuria.

The relative risk (RR) of BV dose per body weight and the occurrence of high-grade proteinuria (grade 3 or grade 4) was reported to be 2.62 (95% CI, 1.61–4.28) with respect to the control group with low-dose BV of 2.5 mg/kg per week, and 8.56 (95% CI, 4.09–17.92) with low-dose BV of 5.0 mg/kg per week. Thus, onset of high-grade proteinuria increased in a dose-dependent fashion (Wu et al. 2010). In another report, the relative risk of BV dose per body weight and the occurrence of proteinuria (grade unknown) was reported to be 1.4 (95%CI, 1.1–1.7) in a low-dose group (BV 3, 5 or 7.5 mg/kg per dose) with respect to the control group, and 2.2 (95%CI, 1.6–2.9) in a high-dose group (BV 10 or 15 mg/kg per dose), increasing in a dose-dependent fashion (Zhu et al. 2007). In the present study, BV dose per body weight showed a significant difference on univariate analysis, but on multivariate analysis, no significant difference was seen after adjusting for confounding factors. The BV dose listed on package inserts differs by type of carcinoma; it is 5 mg/kg,

Table 2: Univariate analysis for each item and the occurrence of proteinuria

Total patients (n = 34)		Non-proteinuria n = 24 (70.6%)	Proteinuria n = 10 (29.4%)	p value
Gender	Male	11 (45.8%)	8 (80.0%)	0.128 [a]
	Female	13 (54.2%)	2 (20.0%)	
Age	≥ 65 years	12 (50.0%)	6 (60.0%)	0.595 [a]
	< 65 years	12 (50.0%)	4 (40.0%)	
BMI	≥ 25	6 (25.0%)	3 (30.0%)	0.763 [a]
	< 25	18 (75.0%)	7 (70.0%)	
Diagnosis [c]	rectal cancer	9 (37.5%)	3 (30.0%)	0.982 [a]
	colon cancer	5 (20.8%)	5 (50.0%)	0.198 [a]
	non-small-cell lung cancer	10 (41.7%)	2 (20.0%)	0.418 [a]
Stage	III	5 (20.8%)	3 (30.0%)	0.666 [a]
	IV	19 (79.2%)	7 (70.0%)	
Dosage (mg/kg)		9.5 ± 4.6 (n = 24)	6.8 ± 3.5 (n = 10)	0.005 [b]
NSAIDs	therapy	3 (12.5%)	1 (10.0%)	0.837 [a]
	non therapy	21 (87.5%)	9 (90.0%)	
Antihypertensive agent	therapy	2 (8.3%)	3 (30.0%)	0.138 [a]
	non therapy	22 (91.7%)	7 (70.0%)	
Multiagent regimen [d]	mFOLFOX	6 (25.0%)	5 (50.0%)	0.232 [a]
	XEROX	7 (29.2%)	1 (10.0%)	0.386 [a]
	PMT	8 (33.3%)	0 (0.0%)	0.072 [a]
	FOLFIRI	1 (4.2%)	1 (10.0%)	0.508 [a]
	CBDCA + TAX	0 (0.0%)	2 (20.0%)	0.080 [a]
	CDDP + PMT	1 (4.2%)	0 (0.0%)	1.000 [a]
	Erlotinib	1 (4.2%)	0 (0.0%)	1.000 [a]
	S-1/L-OHP	0 (0.0%)	1 (10.0%)	0.294 [a]
Systolic blood pressure	≥ 130 mmHg	6 (25.0%))	5 (50.0%)	0.232 [a]
	< 130 mmHg	18 (75.0%)	5 (50.0%)	
Diastolic blood pressure	≥ 85 mmHg	3 (12.5%)	3 (30.0%)	0.328 [a]
	< 85 mmHg	21 (87.5%)	7 (70.0%)	
Biochemical examinatio	Serum sodium (mEq/mL)	137.7 ± 4.7 (n = 20)	139.0 ± 2.1 (n = 7)	0.519 [b]
	Serum potassium (mEq/mL)	4.2 ± 0.4 (n = 20)	4.2 ± 0.3 (n = 7)	0.732 [b]
	Serum creatinine (mg/dL)	0.7 ± 0.2 (n = 24)	0.7 ± 0.1 (n = 10)	0.572 [b]
	Blood urea nitrogen (mg/dL)	12.2 ± 3.2 (n = 24)	13.3 ± 3.3 (n = 10)	0.400 [b]
	Aspartate aminotransferase (IU/L)	25.9 ± 10.4 (n = 24)	25.5 ± 8.0 (n = 10)	0.749 [b]
	Alanine aminotransferase (IU/L)	24.3 ± 16.7 (n = 24)	25.6 ± 10.1 (n = 10)	0.613 [b]
	T-Bil (mg/dL)	0.7 ± 0.7 (n = 24)	0.6 ± 0.2 (n = 10)	0.321 [b]
	Fasting blood sugar level (mg/dL)	106.7 ± 22.7 (n = 9)	148.0 ± 43.1 (n = 4)	0.038 [b]

a) Fisher's exact probability test was used for nominal variables, and b) Student's *t*-test was used for continuous variables. Nominal variables are expressed as numbers (%), and continuous variables are expressed as means ± SD. c) Analysis was done for each of the corresponding diagnoses and all other diagnoses (for rectal cancer, the analysis was done for rectal cancer and other cancers). d) Analysis was done for each of the corresponding combination regimens and all other combination regimens.

7.5 mg/kg, or 10 mg/kg for advanced or recurrent colorectal cancer and 15 mg/kg for non-small-cell lung cancer, but it was not identified as a risk factor in type of carcinoma. With regard to the relationship between proteinuria and BV dose, further investigation of the development of proteinuria with a greater number of patients will be needed in the future.

In an univariate analysis of the relationship between blood sugar level and proteinuria, a significant difference was seen in the

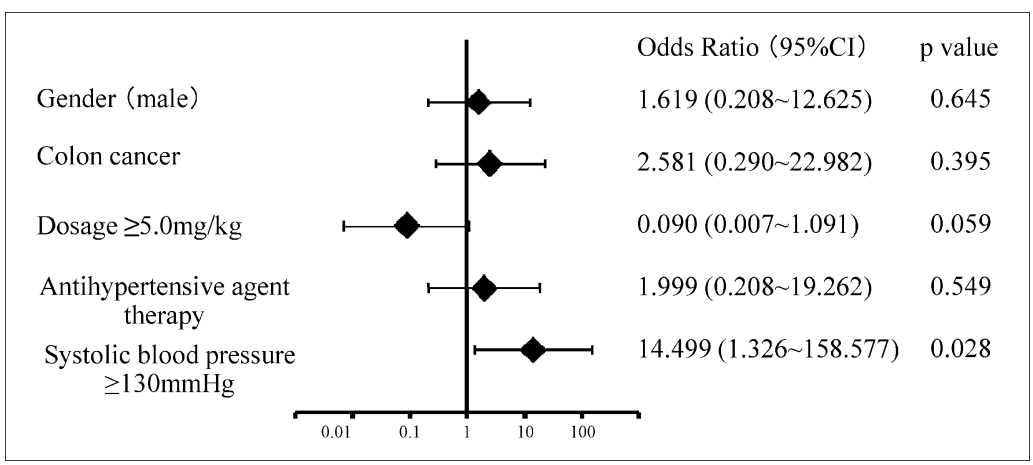

Fig. 2: Multivariate logistic regression analysis for each item and urinary protein expression

blood sugar level. Patients with diabetes are reported to be at risk for proteinuria (Wu et al. 2010), and when administering BV to patients with high blood sugar levels and patients with diabetes, sufficient attention to the development of proteinuria is necessary.

In an univariate analysis of the relationship between type of carcinoma and proteinuria in the present study, no significant difference was seen in type of carcinoma. In a comparison of renal cell cancer and cancers other than renal cell cancer (Wu et al. 2010), the risk of proteinuria was reported to be significantly higher in patients with renal cell cancer, but in Japan, BV has not been approved for renal cell cancer. Currently, there are no reports on the risk of proteinuria in cancers other than renal cell cancer. In the present study as well, no difference was seen in the occurrence of proteinuria in colorectal cancer or lung cancer.

With regard to the relationship between the regimen and proteinuria, Wu et al. (2010) compared platinum-based chemotherapy and other types of chemotherapy, and from the fact that there was no difference in the risk of proteinuria, reported that onset of proteinuria is not greatly affected by the type of regimen. No significant difference was seen in the present study by the type of regimen.

In the present study, systolic blood pressure ($\geq$ 130 mmHg) was shown to be an independent risk factor for the development of proteinuria in BV combination chemotherapy. When systolic blood pressure at the start of BV combination chemotherapy is $\geq$ 130 mmHg, it is suggested that the risk of proteinuria should be reduced and increases in severity of proteinuria prevented through measures such as administration of antihypertensives and appropriate blood pressure management. Further investigation will be needed in the future with respect to dose per body weight.

4. Experimental

4.1. Subjects

The subjects were patients aged $\geq$ 20 years who received BV combination therapy at Gifu Municipal Hospital between February 2010 and February 2011. Patients who met any of the following conditions at the start of chemotherapy were excluded: patients with primary tumors in multiple organs, patients receiving continuous systemic administration (oral or intravenous) of steroids, patients with poorly controlled diabetes, and patients in whom BV combination chemotherapy was discontinued before one cycle was completed.

4.2. Survey items

The following information was gathered retrospectively from electronic health records: patients' sex, age, height, and weight; type of carcinoma and stage classification; combination regimen; BV dose; blood pressure; whether they were taking antihypertensives or NSAIDS; whether they had proteinuria and, if they did, its grade classification; serum electrolytes (Na, K); serum creatinine (Scr); blood urea nitrogen (BUN); aspartate aminotransferase (AST); alanine aminotransferase (ALT); total bilirubin (T-Bil); and fasting blood sugar level. The severity of proteinuria was evaluated using the Common Terminology Criteria for Adverse Events (CTCAE) ver. 3.0.

4.3. Statistical analysis

Statistical analysis was performed using statistical analysis software SPSS 18.0J. Patients were divided into two groups: a no-proteinuria group and a proteinuria group. Using Fisher's exact probability test for nominal variables and Student's *t*-test for continuous variables, an evaluation was performed of whether each factor was significantly related to the occurrence of proteinuria. Dichotomous variables were adopted with age divided into $\geq$ 65 years and < 65 years according to the WHO criteria and BMI divided into $\geq$ 25 kg/m^2 and < 25 kg/m^2 according to the criteria of the Japan Society for the Study of Obesity. A dichotomous variable was adopted for diagnosis, divided into types of carcinoma for analysis subjects and all other types of carcinoma. Similarly, a dichotomous variable was adopted for regimen, divided into regimens for analysis subjects and all other regimens. Dichotomous variables were adopted for blood pressure, with systolic blood pressure divided into $\geq$ 130 mmHg and < 130 mmHg, and diastolic blood pressure divided into $\geq$ 85 mmHg and < 85 mmHg, based on the standards of the 2009 Guidelines for the Management of Hypertension in Japan. A multiple logistic regression analysis was then conducted with the two groups, the no-proteinuria group and the proteinuria group, as response variables. In all cases, $p < 0.05$ was taken to indicate a significant difference.

4.4. Ethical considerations

This study complied with ethical guidelines for clinical research and was approved by the medical research ethical review board of Gifu Municipal Hospital. To protect patients' privacy, personal information was anonymized in a linkable fashion for the data analysis. Patients' personal information could not be identified, and there was no disadvantage to patients.

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