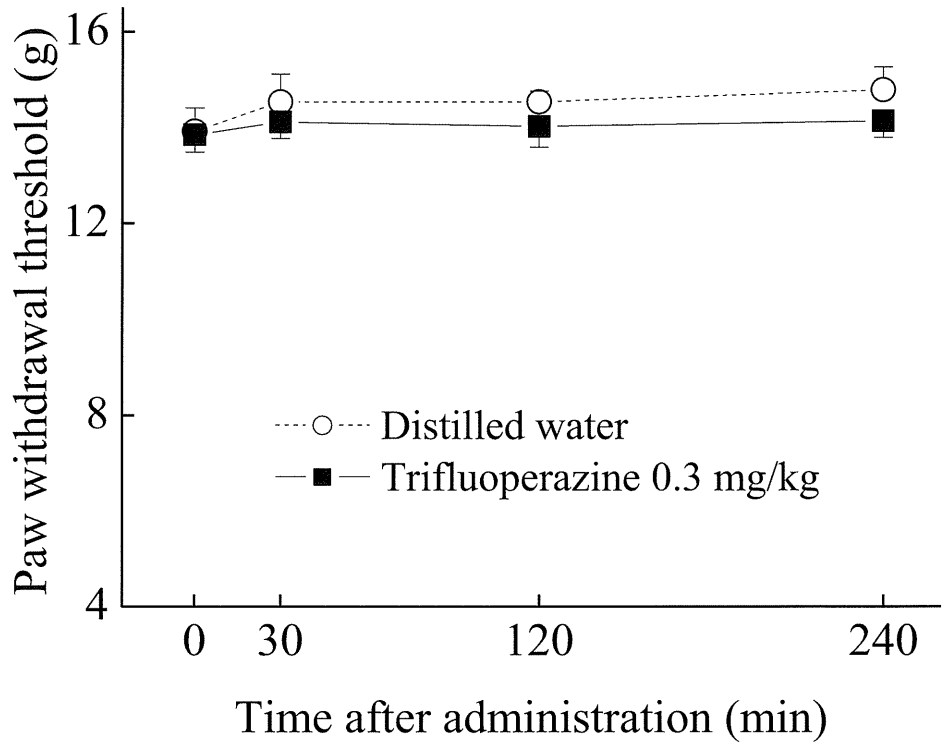

Figure 5 Effect of trifluoperazine on mechanical nociceptive threshold in the von Frey test. Trifluoperazine (0.3 mg/kg) was administered orally in intact rats. The von Frey test was performed immediately before (0 min) and at 30, 120 and 240 min after administration of trifluoperazine. Trifluoperazine did not affect mechanical nociceptive threshold in intact rats. Values are expressed as the mean ± SEM. of 8 animals. No statistical difference was identified (one-way ANOVA followed by Tukey-Kramer post-hoc test).

trifluoperazine on spinal CaMKII activity may be involved in the reduction of pain behavior, and low doses of trifluoperazine may be useful for the treatment of the oxaliplatin-induced neuropathy.

Conclusions

Our results indicate that repeated administration of oxaliplatin increases spinal CaMKII activity. This increase of CaMKII activation was reversed by intrathecal injection of the selective CaMKII inhibitor and the selective NR2B antagonist. This CaMKII activation may contribute to the incidence of mechanical allodynia. Furthermore, the selective CaMKII inhibitor and the selective NR2B antagonist reduced the oxaliplatin-induced pain behavior. In addition, trifluoperazine reduced the oxaliplatin-induced mechanical allodynia and CaMKII activation. These results suggest that inhibition of CaMKII or NMDA-CaMKII pathway provides a novel therapeutic target for the treatment of the oxaliplatin-induced peripheral neuropathy.

Methods

Animals

Male Sprague-Dawley rats weighing 200-250 g (Kyudo Co., Saga, Japan) were used in the present study. Animals were housed in groups of four to five per cage, with lights on from 7:00 to 19:00 h. Animals had free access to food and water in their home cages. All experiments were approved by the Experimental Animal Care and Use Committee of Kyushu University according to the National Institutes of Health guidelines, and we followed International Association for the Study of Pain (IASP) Committee for Research and Ethical Issues guidelines for animal research [23].

Drugs

Oxaliplatin (Elplat®) was obtained from Yakult Co., Ltd. (Tokyo, Japan). KN-93, Ro 25-6981 hydrochloride hydrate and trifluoperazine dihydrochloride were purchased from Sigma-Aldrich (Missouri, USA). KN-92 was purchased from Calbiochem (California, USA). Oxaliplatin was dissolved in 5% glucose solution. The vehicle-treated rats were injected with 5% glucose solution. KN-93, KN-92 and Ro 25-6981 were dissolved in 100% dimethyl sulfoxide (DMSO). Trifluoperazine was dissolved in distilled water. The doses of these drugs were chosen based on previous reports [2,3,7].

Production of neuropathy

Mechanical allodynia and cold hyperalgesia were induced according to the method described previously

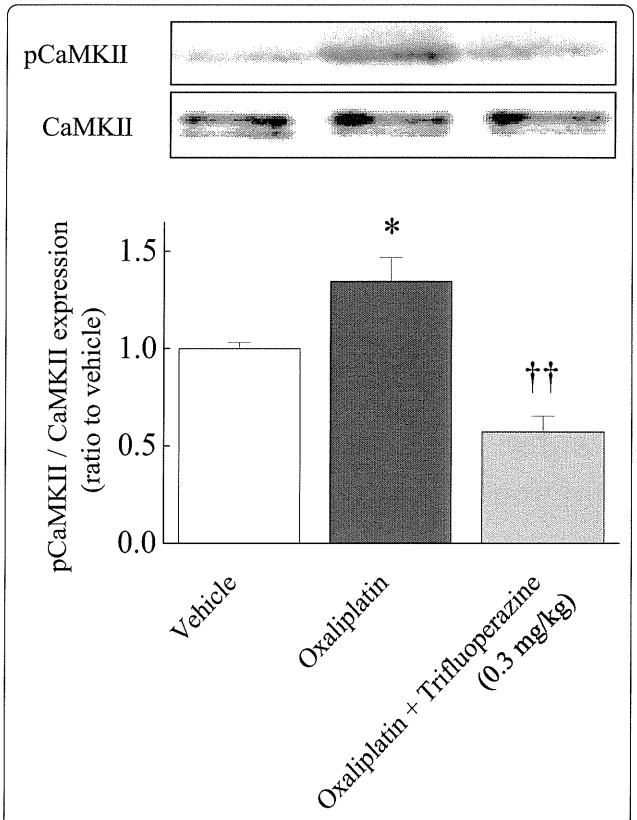

Figure 6 Effect of trifluoperazine on oxaliplatin-induced increase of spinal CaMKII phosphorylation. Rats were treated with oxaliplatin (4 mg/kg, i.p.) twice a week for 4 weeks (days 1, 2, 8, 9, 15, 16, 22 and 23). The lumbar sections (L_{4-6}) of the spinal cord were quickly removed at 30 min after administration of trifluoperazine (0.3 mg/kg, p.o.) on day 25. CaMKII phosphorylation (pCaMKII) in the lumbar sections of the spinal cord was determined by Western blotting. An increase of pCaMKII was found in the spinal cord of oxaliplatin-treated rats. Acute treatment with trifluoperazine reduced oxaliplatin-induced increase in the spinal pCaMKII. Values are expressed as mean ± SEM. of 6-7 animals. *$p < 0.05$ compared with vehicle, ††$p < 0.01$ compared with oxaliplatin alone by Tukey-Kramer post-hoc test.

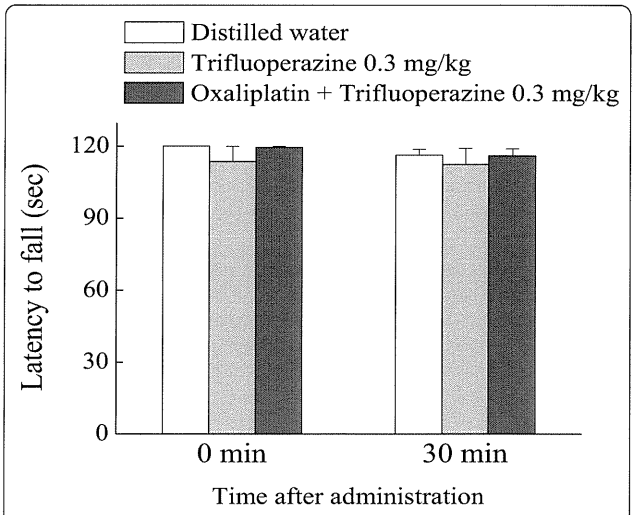

Figure 7 Effect of trifluoperazine on motor coordination in the rota-rod test. Trifluoperazine (0.3 mg/kg) was administered orally in intact and oxaliplatin-treated rats. The rota-rod test was performed immediately before (0 min) and at 30 min after administration of trifluoperazine. Trifluoperazine did not affect motor coordination in intact and oxaliplatin-treated rats. Values are expressed as the mean ± SEM. of 8-11 animals. No statistical difference was identified (one-way ANOVA followed by Tukey-Kramer post-hoc test).

[24]. Oxaliplatin (4 mg/kg) or vehicle (5% glucose solution) was administered i.p. twice a week for 4 weeks (on days 1, 2, 8, 9, 15, 16, 22 and 23). The volume of vehicle or drug solution injected was 1 mL/kg for all drugs.

Behavioral studies

Behavioral test was performed blindly with respect to drug administration.

von Frey test for mechanical allodynia

The mechanical allodynia was assessed by von Frey test. Each rat was placed in a clear plastic box (20 × 17 × 13 cm) with a wire mesh floor and allowed to habituate for 30 min prior to testing. von Frey filaments (The Touch Test Sensory Evaluator Set; Linton Instrumentation, Norfolk, UK) ranging from 1- to 15-g bending force were applied to the midplantar skin of each hind paw six times, with each application held for 6 s. The paw withdrawal threshold was determined by a modified up-down method [25].

Acetone test for cold hyperalgesia

The cold hyperalgesia was assessed by acetone test. Each rat was placed in a clear plastic box (20 × 17 × 13 cm) with a wire mesh floor and allowed to habituate for 30 min prior to testing. Fifty microliters of acetone (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was sprayed onto the plantar skin of each hind paw 3 times, and the number of withdrawal responses was counted for 40 s from the start of the acetone spray.

Rota-rod test for motor coordination

The rota-rod test was performed to investigate the change of motor coordination. Rats were placed on a rotating rod (Muromachi Kikai Co., Ltd., Tokyo, Japan) and the latency to falling was measured for up to 2 min according to the method described previously [26]. The test was performed three times, and the rotating speed was 10 rpm.

Effects of KN-93, KN-92 and trifluoperazine on Oxaliplatin-induced mechanical allodynia

We confirmed the incidence of mechanical allodynia in the von Frey test on day 24. We carried out the drug evaluation on the next day. KN-93 (10-50 nmol) or KN-92 (50 nmol) was administered i.t. injection by direct

lumbar puncture in a volume of 50 μL. The von Frey test was performed immediately before (0 min) and at 30, 60, 90 and 120 min after administration of the drugs. Trifluoperazine (0.05-0.3 mg/kg) was administered p.o. The von Frey test was performed immediately before (0 min) and at 30, 120 and 240 min after oral administration of trifluoperazine.

Effect of KN-93 on Oxaliplatin-induced cold hyperalgesia

We confirmed the incidence of cold hyperalgesia in the acetone test on day 5. KN-93 (10-50 nmol) was administered i.t. injection by direct lumbar puncture in a volume of 50 μL. The acetone test was performed immediately before (0 min) and at 30, 60, 90 and 120 min after administration of the drug.

Effect of trifluoperazine on mechanical nociceptive threshold

We investigated the effect of trifluoperazine on the mechanical nociceptive threshold in the von Frey test. Trifluoperazine (0.3 mg/kg) was administered p.o. in intact rats. The von Frey test was performed immediately before (0 min) and at 30, 120 and 240 min after oral administration of trifluoperazine.

Effect of trifluoperazine on motor coordination

We investigated the effect of trifluoperazine on the motor coordination in the rota-rod test. Trifluoperazine (0.3 mg/kg) was administered p.o. in intact and oxaliplatin-treated rats. The rota-rod test was performed immediately before (0 min) and at 30 min after oral administration of trifluoperazine.

Western blotting analysis

The lumbar sections (L_{4-6}) of the spinal cord were quickly removed at 30 min after administration of KN-93 (50 nmol, i.t.), Ro 25-6981 (300 nmol, i.t.) or trifluoperazine (0.3 mg/kg, p.o.) on day 25. The tissues were homogenized in a solubilization buffer containing 20 mM Tris-HCl (pH 7.4, 2 mM EDTA, 0.5 mM EGTA, 10 mM NaF, 1 mM Na_3VO_4, 1 mM PMSF, 0.32 M Sucrose, 2 mg/ml aprotinine, 2 mg/ml leupeptin), and the homogenates were subjected to 12.5% SDS-PAGE, and proteins were transferred electrophoretically to PVDF membranes. The membranes were blocked in Tris-buffered saline Tween-20 (TBST) containing 5% BSA (Sigma-Aldrich) for an additional 1 h at room temperature with agitation. The membrane was incubated overnight at 4°C with mouse polyclonal anti-CaMKIIα antibody or rabbit polyclonal anti-(Thr286)pCaMKII (1:5000; Santa Cruz Biotechnology, California, USA) and then incubated for 1 h with corresponding horseradish peroxidase conjugate secondary antibodies (1:5000; Jackson Immuno Research Laboratories, Inc., PA, USA). The immunoreactivity was detected using Enhanced Chemiluminescence (Perkin Elmer, Massachusetts, USA). Ratios of the optical densities of pCaMKII to those of CaMKII were calculated for each sample.

Data analysis

Values were expressed as the means ± SEM. The values were analyzed by the Student's *t*-test or one-way analysis of variance (ANOVA) followed by the Tukey-Kramer post-hoc test (StatView; Abacus Concepts, Berkely, CA, USA) to determine differences among the groups. A p value of less than 0.05 is considered as statistically significant.

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Authors' contributions

NE and TK are responsible for experimental design. MS, SU and HS are responsible for performance of behavioral tests. MS, SU, SY, HS and KM are responsible for performance of Western blotting. NE, MS and RO are responsible for writing the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Original article

Calcium channel blockers reduce oxaliplatin-induced acute neuropathy: A retrospective study of 69 male patients receiving modified FOLFOX6 therapy

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ABSTRACT

Oxaliplatin-based chemotherapy has been widely used for colorectal cancer. However, it causes severe acute and chronic peripheral neuropathies. Recently, we reported that calcium channel blockers prevent the oxaliplatin-induced cold hyperalgesia in rats. The purpose of this study was to determine whether the treatment with calcium channel blockers prevents the peripheral neuropathy during oxaliplatin therapy. The electronic medical charts for patients who received modified FOLFOX6 regimen from January 2008 to December 2010 were evaluated. Of the 200 patients who received modified FOLFOX6 therapy, 84 patients were excluded due to the exclusion criteria. Calcium channel blockers had been taken by 26 of 69 male patients, but only three of 47 female patients. Therefore, in the present analysis, the male data of the groups with and without calcium channel blockers ($n = 26$ and 43, respectively) were compared. The cumulative incidence curve of acute neuropathy was significantly lower in the group with calcium channel blockers ($P = 0.0438$, log-rank test), whereas there was no difference between these groups in the cumulative incidence curve of chronic neuropathy ($P = 0.4919$, log-rank test). The present study indicated that calcium channel blockers inhibit the development of acute peripheral neuropathy in patients receiving modified FOLFOX6 therapy.

1. Introduction

Oxaliplatin-based chemotherapy has been widely used for colorectal cancer. However, it causes severe acute and chronic peripheral neuropathies. Acute neuropathy is peculiar to oxaliplatin and appears soon after administration [1–3]. The acute neuropathy occurs in about 85 to 95% of all patients receiving oxaliplatin [4]. The patients suffer from paresthesia in the extremities and perioral area, shortness of breath, swallowing difficulty and in particular from severe cold hypersensitivity enhanced by exposure to cold [1,3–5]. In addition, pharyngolaryngeal dysesthesia, throat and jaw tightness, and dysphonia often occurred [6–8]. It has been thought that the acute neuropathy is not due to morphological damage of the nerve [9] and is due to alternations of voltage-gated Na^+ and K^+ channels [10–13]. In clinical trials, calcium and magnesium infusions have been tried to reduce the oxaliplatin-induced neuropathy [14,15]. In addition, gabapentin is recommended as first-line treatment for the neuropathic pain [16]. However, a phase III randomized double-blind trial failed to demonstrate any benefit to using gabapentin to treat symptoms of chemotherapy-induced peripheral neuropathy [17]. Therefore, new agents to strongly reduce the symptoms of neuropathy are required.

We previously reported that repeated administration of oxaliplatin induced cold hyperalgesia from the early phase and mechanical allodynia in the late phase in rats, and that oxalate derived from oxaliplatin is involved in the cold hyperalgesia [18]. Recently, an increase in transient receptor potential (TRP) melastatin 8 (TRPM8) mRNA levels was reported to be involved in the oxaliplatin-induced cold hyperalgesia in mice [19]. TRPM8 is an ion channel that belongs to the TRP family and it is activated by cold temperatures (< 25 °C) or menthol [20,21]. We also found that treatment with oxaliplatin induced cold hyperalgesia and the increase in TRPM8 mRNA levels via Ca^{2+} influx in cultured rat dorsal root ganglia [22]. Interestingly, co-administration with calcium channel blockers such as nifedipine prevents the oxaliplatin-induced cold hyperalgesia in rats [22].

Calcium channel blockers are commonly-used drugs for controlling blood pressure. However, there is little published data regarding the influence of calcium channel blockers on the incidence of peripheral neuropathy during oxaliplatin treatment. We, therefore, investigated to determine whether the treatment

Abbreviations: TRP, Transient receptor potential; TRPM8, Transient receptor potential melastatin 8.

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with calcium channel blockers prevents the peripheral neuropathy in patients receiving oxaliplatin therapy.

2. Materials and methods

2.1. Patients

All patients who were administered oxaliplatin from January 2008 to December 2010 at Kyushu University Hospital were identified and their electronic medical charts were evaluated. Patients with known peripheral neuropathy, brain metastasis, prior oxaliplatin-containing chemotherapy and oxaliplatin-based chemotherapy except modified FOLFOX6 were excluded. Patients treated with opioids, gabapentin, gosha-jinki-gan and vitamin B_{12} were also excluded because these drugs have been reported to ameliorate the various neuropathies [23–26]. The present study was conducted in accordance with the Declaration of Helsinki and its amendments, and the protocol was approved by the ethics committee of Faculty of Medicine, Kyushu University (approved no. 22-147 of the institutional review board).

2.2. Chemotherapy

Patients received modified FOLFOX6 regimen: comprising oxaliplatin 85 mg/m^2 and *l*-leucovorin 200 mg/m^2 administered as 2-h infusions on day 1, followed by a 5-fluorouracil bolus of 400 mg/m^2 and a 46-h infusion of 5-fluorouracil 2400 mg/m^2 over days 1 and 2. The chemotherapy was repeated once every two weeks and was continued unless the disease progression, development of severe side effects, refusal of care, or decision of discontinuation of treatment by physician.

2.3. Evaluation criteria

Chronic neuropathy is cumulative and is most commonly seen in patients who received oxaliplatin at the total doses of more than 540 mg/m^2 [27]. Additionally, cisplatin, which induces peripheral neuropathy like oxaliplatin-induced chronic neuropathy, often induces neuropathy at the cumulative dose of 350 mg/m^2 [28]. As an acute neuropathy, the acute neurotoxicity symptoms such as severe cold hypersensitivity of limbs, perioral paresthesias, shortness of breath, swallowing difficulty, pharyngolaryngeal dysesthesia, throat and jaw tightness and dysphonia in the first four cycles of modified FOLFOX6 (cumulative dose under 340 mg/m^2) were extracted from the electronic medical charts. Since the National Cancer Institute-Common Toxicity Criteria was inappropriate for the evaluation of acute neuropathy symptoms, we evaluated incidence of symptoms. Since chronic neuropathy is the main dose-limiting toxicity of oxaliplatin, we captured the change of chemotherapy schedule and/or addition of supplementary analgesics as the surrogate endpoint of chronic neuropathy.

2.4. Statistical analysis

Data were analyzed retrospectively for the association of use of calcium channel blockers and the occurrence of acute neuropathy due to modified FOLFOX6. The incidence of acute neuropathy was evaluated in patient subgroups treated with or without calcium channel blockers at baseline in patients who received modified FOLFOX6. Kaplan-Meier curves were constructed to show the probability of acute neuropathy in relation to increasing cumulative dose of oxaliplatin, and log-rank test was used for evaluation of the differences in the curves. For the comparison of distribution of samples, data were examined using Mann-Whitney *U* test, Fisher's exact test and χ^2 test with Yate's correlation as appropriate. *P* value of < 0.05 was considered as statistically significant. All statistical analyses were carried out using Stat view (Abacus Concepts, Berkeley, CA, USA).

3. Results

A consort diagram is presented in Fig. 1. Between January 2008 to December 2010, a total of 200 patients were treated with modified FOLFOX6. Of these, 84 patients were excluded due to the exclusion criteria. Calcium channel blockers had been taken by 26 of 69 male patients, but only three of 47 female patients. Therefore, in the present analysis, the male data of the groups with and without calcium channel blockers ($n = 26$ and 43, respectively) were compared. Although patients who received calcium channel blockers (calcium channel blocker group) were older than those without these drugs (control group) (median age 70 versus 62 years, respectively, $P = 0.0015$), the demographic characteristics of the calcium channel blocker group significantly did not differ from control group for the rest (Table 1). All patients of calcium channel blocker group were chronically treated with calcium channel blockers before the start of oxaliplatin therapy. Calcium channel

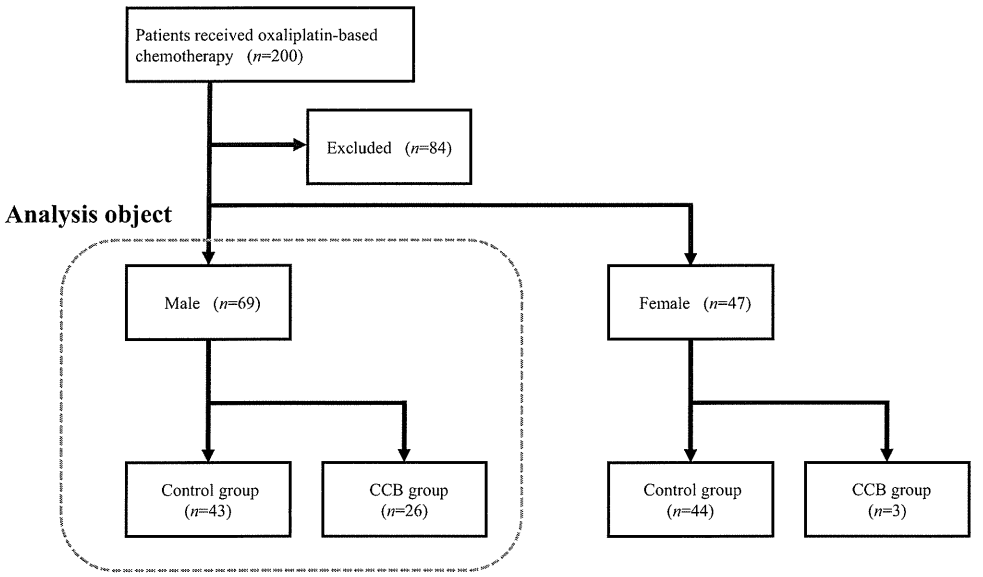

Fig. 1. Consort diagram. CCB: calcium channel blockers.

Table 1
Patients characteristics.

	Control group $n=43$	Calcium channel blocker group $n=26$	P value
Age (year)			
Median (range)	62 (36–83)	70 (42–84)	0.0015[a]
Primary tumor n (%)			
Colorectal	39 (91)	22 (85)	0.4637[b]
Others	4 (9)	4 (15)	
Diabetes n (%)			
With	6 (14)	9 (35)	0.0855[c]
Without	37 (86)	17 (65)	
Relative dose intensity of oxaliplatin (%)			
Median (range)	89 (47–102)	88 (47–98)	0.5731[a]
Prior chemotherapy n (%)			
Yes	11 (26)	6 (23)	>0.9999[c]
No	32 (74)	20 (77)	
Surgery of primary tumor n (%)			
Yes	34 (79)	21 (81)	>0.9999[c]
No	9 (21)	5 (19)	

[a] Mann-Whitney U test.
[b] Fisher's exact test.
[c] χ^2 test with Yates' correction.

blockers used in these patients were amlodipine, nifedipine, azelnidipine, diltiazem, benidipine, cilnidipine, nilvadipine (Table 2).

The incidence of acute neuropathy increased with increasing cumulative dose of oxaliplatin (Fig. 2). The cumulative incidence curve of acute neuropathy was significantly lower in the calcium channel blocker group ($P = 0.0438$, log-rank test, Fig. 2A), whereas there was no difference between these groups in the cumulative incidence curve of chronic neuropathy ($P = 0.4919$, log-rank test; Fig. 2B).

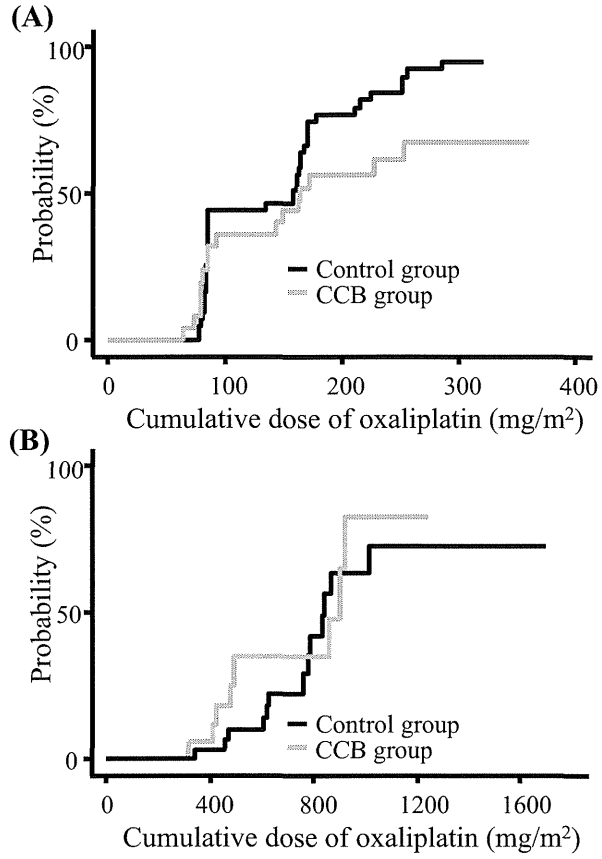

Fig. 2. Probability of acute (A) and chronic (B) neuropathy by cumulative dose of oxaliplatin in patients treated with or without calcium channel blockers.

4. Discussion

In this study, the cumulative incidence curve of acute neuropathy was significantly lower in the calcium channel blocker group, whereas there was no difference between these groups in the cumulative incidence curve of chronic neuropathy. Thus, this retrospective analysis indicates for the first time that the calcium channel blockers inhibit the developing of acute but not chronic neuropathy in patients receiving modified FOLFOX6. Oxaliplatin is metabolized to oxalate and platinum metabolites such as dichloro(1,2-diaminocyclohexane)platinum [29]. In the study using rats treated with oxaliplatin, we demonstrated that oxalate and platinum metabolites are involved in the cold hyperalgesia from the early phase and mechanical allodynia in the late phase, respectively [18]. Furthermore, our data suggested that calcium channel blockers have prophylactic potential for acute neuropathy [22]. Our present findings are in good agreement with the results from the experimental models [22].

Table 2
Breakdown of calcium channel blockers.

	n (%)
Amlodipine	12 (46)
Nifedipine	6 (23)
Azelnidipine	2 (8)
Diltiazem	2 (8)
Benidipine	1 (4)
Cilnidipine	1 (4)
Nilvadipine	1 (4)
Amlodipine and nilvadipine	1 (4)

The chronic neuropathy is characterized by loss of sensory and motor neuropathy after long-term treatment of oxaliplatin and it is similar to cisplatin-induced neurological symptom [1]. Recently, we reported that repeated administration of oxaliplatin causes the degeneration and the decrease in the density of myelinated fibers in rat sciatic nerve in late phase but not early phase [9]. Thus, the mechanism underlying chronic neuropathy seems to be different from that of acute neuropathy.

In the present study, we evaluated the data of males only because female patients, who had taken calcium channel blockers, were a few. Gamelin et al. [30] have reported that the oxaliplatin-induced neurotoxicity was caused equally in men and women, but women seemed to have more severe neuropathy. In general, females exhibit lower thresholds, greater ability to discriminate, higher pain ratings, and less tolerance of noxious stimuli than males [31]. As a result, we could exclude the sexual influence in this study.

In the present analysis, calcium channel blocker group was older than control group. Perhaps, the reason is that the use of calcium channel blockers is related to advanced age. Since age is not a risk factor of oxaliplatin-induced neuropathy [32,33], the influence of age is unlikely to have a significant impact on the present results. However, the prospective studies need to be done to confirm the influence of age.

Currently, the addition of anti-angiogenic drug bevacizumab to oxaliplatin-based chemotherapy is commonly conducted in first-line chemotherapeutic treatment to enhance the effect of oxaliplatin. Since bevacizumab often induces hypertension as an adverse effect, antihypertensive drugs are used for its treatment [34]. In addition, calcium channel blockers have no interaction with oxaliplatin. Indeed, there is no report to indicate the calcium

channel blockers affect the antitumor activity or side effects of oxaliplatin. In light of our finding, calcium channel blockers may be adequate for treatment of hypertension in patients receiving oxaliplatin therapy.

In conclusion, this retrospective analysis indicates that calcium channel blockers inhibit the development of acute neuropathy in patients receiving modified FOLFOX6. However, it was difficult to properly regard the grade of the neuropathy since this study was a retrospective study. Therefore, appropriately powered prospective studies are required to confirm an unequivocal application of calcium channel blockers as a preventive agent against acute neuropathy in patients receiving oxaliplatin therapy. We recommend that investigators prospectively collect data regarding preventive effects of calcium channel blockers on the oxaliplatin-induced acute neuropathy.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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臨床研究 I

XELOX＋Bevacizumab療法におけるチーム医療の実践

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進行大腸癌化学療法であるXELOX±BV療法の有効性・安全性を確認するための第Ⅱ相臨床試験を計画した．[方法] 主評価項目は奏効率，副次的評価項目は無増悪生存期間，安全性(手足症候群発生割合)，治療成功期間とした．本試験では全例に対し医師，看護師，薬剤師によるチームで副作用対策に取り組んだ．この結果，副作用発現率の低下と相対的用量強度の維持に有効であったため報告する．

国内Ⅰ/Ⅱ相試験であるJO19380試験での手足症候群（HFS）発現率はgrade2/3が17.2%/1.7%であったが当院では13.3%/0%と良好な結果であった．相対的用量強度は6コース時点でL-OHP 89.2%，Xeloda 91.0%で良好であった．またその効果はCR/PR/NC/PD割合がそれぞれ10%/56.7%/16.7%/3.3%で奏効率66.7%，病勢制御率96.7%と満足できる結果であった．[結語] 今後も増加するであろう外来での抗癌剤治療では，自宅での管理がより一層重要となる．チームでの取り組みは今後更に必要になると考えられる．

索引用語：チーム医療，XELOX療法，大腸癌化学療法

はじめに

近年，チーム医療の推進が重要なキーワードとして注目され，栄養や感染制御，褥創サポートチームなどが運用されている．しかし，がん領域においては，実臨床現場で具体的なプランとして十分に機能し患者に提供されているとはいい難く，いまだ模索状態といえる．われわれは，新しい進行大腸癌のregimenであるXELOX±bevacizumab（BV）療法の患者を対象に，有効性・安全性を評価する臨床第Ⅱ相試験を計画した．主評価項目を奏効率（Response Rate RR）とし，副次的評価項目は無増悪生存期間(progression free survival PFS)，安全性(手足症候群の発生割合)，治療成功期間(time to treatment failure TTF）とした．当院の疫学・臨床研究など倫理審査委員会にて承認されている．この試験では全例に医師，看護師，薬剤師によるチームを結成し，副作用対策・指導を行った．この結果，副作用発現率の低下と，安易な減量・休薬を避け相対的用量強度（relative dose intensity RDI）を上げる効果を認めたため奏効率とともに報告する．

対象・方法

2009年11月より2011年4月までに男性22名，女性10名の合計32例の進行再発大腸癌初回治療例の登録があった．このうち投与前に転居し投与されなかった1例と，登録後転移巣が原発性肝腫瘍と診断しなおされ，投与されなかった1例を除いた30例で検討した．患者背景を別に示す(表1)．チームアプローチでは医師，看護師，薬剤師が副作用対策にかかわり，手足症候群の軟膏治療やステロイド剤の追加ポイント，電話連絡によるコンプライアンス確認と副作用発現状況の確認を行い，次回の外来受診時に十分な対応ができるようにした．

本チームの各担当者の役割を示す（図1）．

[医師の役割] 臨床試験のインフォームドコンセントをとり，チームの担当者へ紹介する．外来受診時には骨髄抑制のチェックと病勢のチェックを行うと同時に手足症候群（hand foot syndrome HFS）のgradingを確認する．

[看護師の役割] 初回治療前よりのケアについてパンフレットやアトラス写真を見せながら説明し，

表1　患者背景

患者背景	XELOX+BV(n=32)	
	例数	%
性別		
男性	22	68.7
女性	10	31.3
年齢		
中央値（範囲）	64.3 (37-76)	
ECOG PS		
0	12	37.5
1	10	31.3
原発部位		
結腸	18	56.3
直腸	14	43.8
転移部位		
肝	16	50
肺	11	34.4
リンパ節	8	25
その他	5	15.6
病変臓器数		
1	15	46.9
2	12	37.5
3	3	9.4
>3	2	6.3
術後補助療法		
あり	9	28.1
なし	23	71.9

実際に手足への軟膏塗布を家人を交えて実践する．手袋の使用や化粧品の指導も行う．特にパートナーへの説明を重視している．外来受診時には，末梢神経障害とHFSについてのアンケート聴取とともに塗り方の再指導やHFSのgradingの確認を行う．この際，特に足に関しては医師の診察時には観察が不十分になることが多いため入念に観察を行う．また，初回投与前に患者の仕事や日常生活動作を聞き取り，週に1回，日勤帯の時間の範囲で患者の自宅などへ電話連絡を行う．その時点でのHFSの程度や食欲，下痢，胃腸障害などの聞き取りをして，簡単な指導を行う．緊急性がある場合は医師に連絡し，受診を指示する場合もある．

[薬剤師の役割] 初回治療時にパンフレットを用いた治療スケジュールの説明をして，本試験では補助治療薬を初めからすべて処方する（図2）ので，薬の薬効の説明使用タイミングの説明を行う．また，Xeloda®の服薬コンプライアンスの確認目的の手帳配布と書き方の説明を行う．外来受診時には手帳の確認とともにHFS，胃腸障害の確認を行う．HFSでは，特にgrade2に進まないように早期のstrong, very strong steroidの使用を患者に指導している．また，末梢投与症例における血管痛対策でのステロイド量の調節を行っている．特に下痢などの胃腸障害に関しては，本療法を受ける患者の多くは大腸癌術後であるため，酸化マグネシウムやパントシン®，大建中湯®などを服用していることが多い．そのため下痢対策で使用するタンナルビン®やロペミン®などと作用が反する薬を持つこととなり，混乱を招く可能性があるためそれぞれの薬効をしっかり説明し，患者自身が症状に合わせて選択できるような教育態勢を整えた．

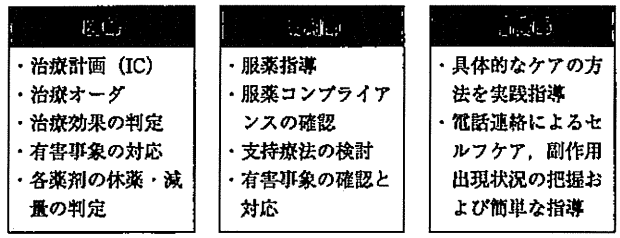

図1　各担当の役割

3者は毎週1回ミーティングを開いて，看護師の連絡によって得られた情報や，薬剤師のラウンドによって得られた情報を共有し，副作用発現時期を予見し，処方医に注意を促したり，副作用対策の投薬の指示を行った．更にHFSのgradingを撮影した写真を見ながら認識を共通化するように検討した．

効果はRECIST規準に則り，安全性に関してはNCI CTCAE ver4.0で評価した．用量強度に関しては，4，6，8コースで算出した．

結　果

30名中継続困難は4例（13.3%）で，初回投与直後からのgrade3の下痢により入院を要し継続困難と判断された1例，HFSはgrade2であったが，試験治療同意撤回となった1例，下痢，倦怠感により主治医により継続困難と判断された2例であった．減量は12例(40.0%)に認め，このうち5例(16.7%)に2段階減量を行った．

[総合評価] CR 3例（10%），PR 17例（56.7%），NC 5例（16.7%），PD 1例（3.3%）でRR 66.7%，病勢制御率(disease control rate DCR)96.7%であった（表2）．

[有害事象] HFSは全gradeでは23例（76.7%）で発現しているが，grade1が20例(66.7%)，grade2

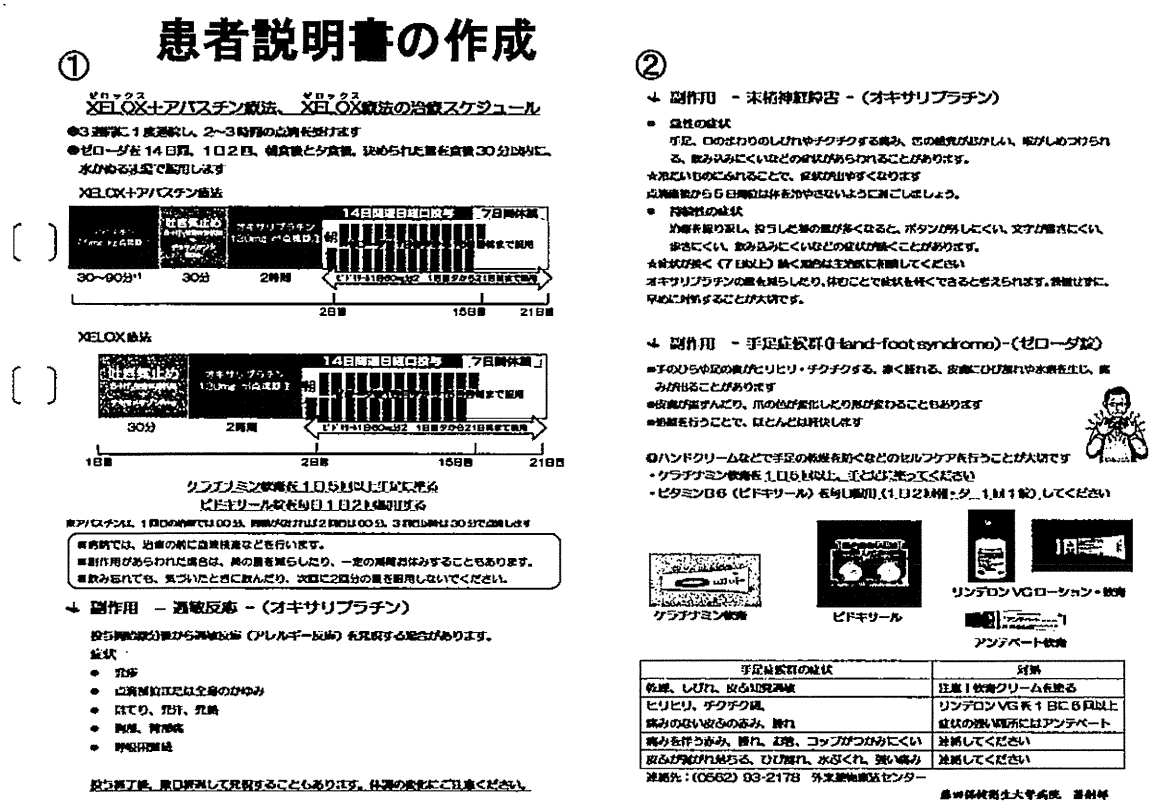

図2　薬剤師の用いる患者説明書

表2　総合評価

RECIST	人数	%
CR	3	10
PR	17	56.7
NC	5	16.7
PD	1	3.3

表3　有害事象

	XELOX＋BV 療法 (n＝32)			
	all grade		grade3-4	
	例数	%	例数	%
手足症候群	23	76.7	0	0
末梢神経障害	19	59.4	3	10.0
下痢	20	62.5	4	13.3
悪心嘔吐	15	46.9	1	3.1
好中球減少	5	15.6	0	0
血小板減少	5	15.6	0	0
疲労	21	65.6	1	3.1
高血圧	7	21.9	0	0
消化管穿孔	1	3.1	1	3.1
尿蛋白陽性	8	25	0	0

表4　相対的用量強度

	4コース	6コース	8コース
XELODA	91.2	91	83.3
L-OHP	92.4	89.2	66.6

が3例（13.3%）でgrade3以上は認めなかった．爪囲炎は1例に認めた．下痢はgrade3が4例（13.3%），grade2が4例（13.3%）であったが，下剤・整腸剤の休薬や減量，あるいは追加の止痢剤にて多くは対応可能であった．骨髄抑制は1例で，grade2の血小板減少でXeloda®，L-OHPともに1段階の減量を行った．末梢神経障害はgrade3を3例（10.0%）に認めた．いずれの例も7コースであった．BVに関連する有害事象としては1例（3%）に高血圧薬剤の追加を認め，1例（3%）タンパク尿の悪化，1例（3%）に原発巣口側の穿孔による緊急手術が行われたが，腫瘍潰瘍部ではなく，大腸イレウスの状態でもなかったため，因果関係は不明であった．有害事象をまとめたものを表に示す（表3）．

［用量強度］Xeloda®とL-OHPそれぞれのRDIを計算すると，Xeloda®は4，6，8コースでそれぞ

れ91.2%，91.0%，83.3%であり，L-OHPはそれぞれ92.4%，89.2%，66.6%であった（表4）．

考　察

2009年10月より進行再発大腸癌に対して保険適用となったXeloda®+L-OHPによるXELOX療法は，これまでの進行再発大腸癌の中心regimenであるFOLFOX療法と同等の成績を持ちながら様々なメリットがあり，急速に処方を伸ばしている[1-6]．そのメリットは，1．ポートフリーの可能性，2．外来通院間隔の延長，3．病院滞在時間の短縮，4．ポンプフリーによる家庭での注射治療がなくなる，5．若干の医療費の軽減などである．一方，この療法ならではの副作用対策が必要である．その一つがHFSで日本での国内第Ⅰ/Ⅱ相臨床試験であるJO19380試験[7]では78%に出現していた．また，他の副作用では下痢が55%に認められていた．一方，重篤な副作用につながる骨髄抑制は52%とFOLFOX療法にくらべて低い傾向であった．骨髄抑制は予防手段がなく，感染症の併発が時に致死的な合併症となる場合があり，現在の外来通院治療が中心の抗癌剤治療においては避けたい副作用である．本療法はその意味でも外来治療の大きなメリットと考えられる．今回のわれわれのデータではHFSは全体の発生割合では76.7%とあまり変わらなかったが，JO19380試験のgrade2/3が17.2%/1.7%であったが当院では13.3%/0%とgrade2，3の発生は少なく，HFSの悪化による延期や減量は1例のみであり，看護師によるケアの実践や薬剤師による早期のstrong, very strong steroidの使用推奨が功を奏していると考えられた．一方で下痢に関してはgrade3が3例10%と多く認められた．これらのうち2例に関してはXELOX療法中止後FOLFOXに変更したところ問題なく継続可能であったため，L-OHP 130mg/mm^{2}の投与量が何らかの影響を与えていると考えられた．RDIでは海外の1st lineのphase Ⅲ試験であるNO16966試験では，XELOXのRDIはXeloda®が75%でL-OHPが84%であった．2nd lineのphase Ⅲ試験であるNO16967試験においても，75%，87%という結果であった．JO19380試験では，74%と86%であった．本試験ではXeloda®とL-OHPそれぞれのRDIを計算すると，Xeloda®は4，6，8コースでそれぞれ91.2%，91.0%，83.3%であり，L-OHPはそれぞれ92.4%，89.2%，66.6%であった．いずれの数値も良好であった．L-OHPの8コースでの低下は計画的休薬によるものであった．一般的にXeloda®による減量の理由はHFSあるいは胃腸障害であり，副作用対策により改善が期待できる部分ともいえる．すなわち，補助治療のよしあしで副作用の発症率が変わる可能性があるため，われわれは主に自宅での副作用対策を行えるような患者指導をチーム編成をして対応するようにした．これによりRDIが高まり，結果としてPFSが延長することを期待される．全観察期間終了後に報告予定である．試験のprimary endpointはRRである．今回の集計ではCR 3例（10%），PR 17例（56.7%），SD 5例（16.7%），PD 1例（3.3%）でRR 66.7%，DCR 96.7%であった．これはJO19380試験とほぼ同等な結果であり，満足できる結果であった．これまで，XELOX療法においては適切な減量，休薬をする方が治療成績を向上させるという報告が散見されるが[8]，今回の結果では十分なチームアプローチを行うことで，患者自身が副作用をコントロール可能であり，コンプライアンスの向上につながった可能性があり，QOLやPSを下げないで治療強度を維持した場合の治療成績については，今後PFSやTTF集積時にRDIの上昇が影響を与えたかどうか検証予定である．

一方，チームで対応することのメリットとしては，情報量の増加があげられる．チームでの役割は先に示したとおりである．チームのミーティングの結果はカルテコンピューター上に記載され，ミーティングに参加していない担当医でも次回診察時にこの3週間の情報が得られることになり，副作用のつらかった時期や，その対策について患者と話しができるようになった．患者としてもきちんと情報が共有化されていることが確認でき，非常に喜ばれている．特に手指足趾関節面での亀裂や軽度の炎症はコース投薬後休薬中に起こることが多く，電話連絡や診察時の事前指導により，早期にステロイドの使用を推奨し潰瘍形成は起こらなかった．今回の電話連絡では，患者自身の判断で休薬するべきなのか補助薬剤で対応するのか判断できなかったケースが散見され，電話連絡がなければ分からなかったという意見も聞かれた．また，受診日のみの診察では休薬により軽快したあとのため，最悪のgradeの観察は困難であると思われた．

外来中心での治療は受診日以外の自宅治療が重要であり，この間のケア不足やコンプライアンスの低下は投薬環境に直結し，ひいては治療の継続性や効果に最も影響がでる部分である．このためにも，今回の取り組みは本療法以外でも外来化学療法の新しい形であるともいえる．もっとも，現状においては電話連絡には保険点数が付いておらず，電話回線が一つつぶれる点や電話使用料，今後患者数の増加や他regimenの追加による看護師の仕事量増加による負担もあり，解決すべき問題もある．症例を重ねることにより効果的な電話のタイミングを再考したり，患者が指導なくすべて自己ケアが確立するポイントを決定することにより効率を高め，医療者の負担の軽減はできると考えられる．今後，抗癌剤治療は更に外来中心になり，更なるチームアプローチが重要になると考えられる．当院での取り組みはこの第一歩として有意義であると思われる．

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Team Approach for XELOX+Bevacizumab Therapy

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XELOX+Bevacizumab (BV) is one of the most common regimens for advanced colorectal cancer in Europe and the US, but there is little clinical data in Japan.

We studied the effectiveness and safety of XELOX+BV therapy for advanced colorectal cancer patients in a phase Ⅱ clinical trial. The primary endpoint was response rate (RR). Secondary endpoints were progression-free survival (PFS), time to treatment failure (TTF) and incidence of adverse events.

In this study we used the team approach for management of adverse events. This report describes the effectiveness of adverse event management and the improvement of ingestion compliance by the team of doctors, nurses, and pharmacists.

The rate of Hand Foot Syndrome grade 2/3 in a domestic phase Ⅰ/Ⅱ study JO19380 was 17.2%/1.7% respectively, while that in our study was 13.2%/0%.

The relative dose intensity of six courses was 89.2% (L-OHP) and 91.0% (XELODA), respectively. The response rate was 66.7%, and the decrease control rate was 96.7%.

Outpatient chemotherapy will increase gradually, and so it will become even more important to control adverse events at home.

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肛門扁平上皮癌に対する化学放射線療法の治療経験

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Summary

We reviewed the clinical records of 6 cases with anal squamous cell carcinoma to evaluate the clinical effectiveness of chemoradiotherapy (CRT). The radiotherapy consisted of 40 Gy delivered to the pelvis and bilateral inguinal lesion, and a perianal booster dose of 20 Gy, in fractions of 2.0 Gy per day, 5 days per week. 5-FU and mitomycin C were administrated 3 times every 4 weeks as standard chemotherapy. On the first day of radiation therapy, 750 mg/m^2 of 5-FU in the form of a continuous 24-hour infusion for 5 days was given. On the first day of chemotherapy, 10 mg/m^2 of mitomycin C was also given as a single bolus infusion. One aged patient with a T3 tumor was administrated oral S-1 during radiotherapy. Four patients had a T2 tumor, 1 had a T1 tumor, and 1 had a T3 tumor. One patient had metastases in the Virchow lymph node that originated from synchronous vaginal cancer. No patient had hematogenous metastases. Grade 2 adverse effects occurred in 3 patients, and Grade 3 in 1 patient, during CRT, but the completion of CRT was achieved in all 6 patients. All patients had complete response (CR) in the anal lesion after CRT. Only the patient with a T3 tumor who was administrated S-1 showed signs of recurrence in the anal lesion. CRT is expected to be a safe and effective treatment for improving the prognosis of anal squamous carcinoma. Key words: Anal cancer, Chemoradiotherapy, 5-fluorouracil

要旨　肛門扁平上皮癌に対して，化学放射線療法（chemoradiotherapy: CRT）を施行した6例の治療成績を検討した。放射線療法（RT）は，小骨盤腔と両側鼠径部に40 Gy/30回照射後，肛門部に20 Gy/10回照射した。RT開始日から5-FU 750 mg/m^2/dayをday 1～5持続静注し，mitomycin C 10 mg/m^{2}をday 1に静注し，4週間ごとに3コース施行する化学療法を標準治療とし，高齢のT3症例ではS-1（40 mg/日）を内服した。腫瘍サイズはT1: 1例，T2: 4例，T3: 1例で，T1症例は同時性膣癌によるVirchowリンパ節への転移を認めたが，5例はリンパ節転移を認めなかった。全例で血行性転移は認めなかった。CRT中3例にGrade 2，1例にGrade 3の有害事象を認めたが，RTの中断や化学療法の開始を1週間以上遅らせることなく全例でCRTを完遂できた。CRTの効果は肛門病変に関して全例がcomplete response（CR）であった。S-1を内服したT3症例を除く5例は再発なく経過観察中である。肛門扁平上皮癌に対するCRTは安全に施行が可能で，根治が期待される治療法と考えられた。

はじめに

肛門扁平上皮癌はhuman papilloma virus，human immunodeficiency virusとの関係が指摘され，近年では増加傾向にあるとされている[1]。1970年代までは欧米においても手術療法が肛門扁平上皮癌に対する治療の中心であったが，現在では化学放射線療法（chemoradiotherapy: CRT）が標準治療として確立されている[2]。近年では，本邦においても肛門扁平上皮癌に対してCRTが施行される頻度は増加傾向にあると考えられるが[3]，まれな疾患であり，その治療成績の報告は少ない。今回，肛門扁平上皮癌に対して初回治療としてCRTを行った6例の治療成績を報告する。

I. 対象および方法

1. 対　象

2012年5月までに経験した肛門扁平上皮癌のうち，初回治療としてCRTが施行された6例を対象とした（表

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表 1 化学放射線療法を施行した肛門扁平上皮癌症例

年齢	性別	T	N	M	stage	重複癌	MMC (mg)	5-FU (mg)	S-1 (mg)	放射線 (Gy)	副作用 (Grade 2以上)	局所効果	再発	局所再発	転帰
59	男性	2	0	—	II	—	14	1,000	/	60	WBC, Plt 減少	CR	−	−	生
83	女性	3	0	—	II	—	/	/	40	60	—	CR	+	+	死
43	女性	1	0	—	I	膣癌	14	1,000	/	40	WBC 減少, 食思不振	CR	−	−	死
36	女性	2	0	—	II	悪性リンパ腫, 咽頭癌	15	1,125	/	60	—	CR	−	−	生
59	女性	2	0	—	II	—	16	1,200	/	60	WBC 減少, 食思不振	CR	−	−	生
59	女性	2	0	—	II	子宮頸癌	14	1,025	/	60	WBC, Plt 減少, 膀胱炎	CR	−	−	生

1)。男性1例, 女性5例で, 平均年齢は56.5 (36〜83) 歳であった。腫瘍サイズはT1: 1例, T2: 4例, T3: 1例, T1の1例では治療開始前のCT検査で, 同時性に合併した膣癌による大動脈周囲および左鎖骨上窩へのリンパ節転移が確認された。また, 6例中3例で同時性または異時性の重複癌を認めた。

2. 化学放射線療法

放射線療法(RT)は総線量を60 Gyとし, 1回線量2.0 Gyを30回に分割して週5回照射した。小骨盤腔と両側鼠径部に前後対向2門照射で20回照射した後, 肛門部に4門照射または回転照射で10回照射した。しかし, T1症例では重複癌による遠隔リンパ節転移を認めること, 肛門癌による症状を認めないことから総線量を40 Gyとした。化学療法は標準治療として放射線照射の開始に合わせて5-FU 750 mg/m^2/day (days 1〜5), mitomycin C (MMC) 10 mg/m^2 (day 1) を投与し, 休薬を含めて4週間を1コースとした。T3症例は83歳と高齢であり, 放射線治療日 (5 day/week) にS-1 40 mg/dayを内服した。

3. 評価方法

CRTの副作用および治療効果について検討した。肛門癌に対する治療効果の評価は造影CT検査, 大腸内視鏡検査, 直腸診を行い, 臨床的にcomplete response (CR) と診断された場合には, 肛門癌の存在した部位の組織を生検して病理学的に腫瘍の遺残の有無を確認した。病期分類はTNM分類第7版[4]に従い, 他の所見の記載は大腸癌取扱い規約第7版補訂版に従った[5]。CRTの治療効果判定はRECISTに準じて行い, 病理学的効果は大腸癌取扱い規約第7版補訂版[5]に従った。有害事象の判定はNational Cancer Institute-Common Toxicity Criteria (NCI-CTC) ver. 3.0に準じて判定した。

II. 結 果

CRT中, 3例にGrade 2, 1例にGrade 3の有害事象を認めた。その内容は食欲不振 (Grade 2を2例), 血小板減少 (Grade 2を1例, Grade 3を1例), 好中球減少 (Grade 2を3例, Grade 3を1例), 膀胱炎 (Grade 2を1例) であった。しかし, RTの中断や化学療法の開始を1週間以上延期することなく全例が治療を完遂した。総線量が40 Gyであった1例を含む全例で化学療法を3または4コース終了した後に, 臨床的または病理学的に肛門癌のCRが確認された。また, CRTの開始から局所病変のCRが確認されるまでに新たな遠隔転移や, リンパ節転移を認めた症例はなかった。

83歳のT3症例は腫瘍からの出血に対してS状結腸人工肛門造設術後にCRTを開始した。CRT終了1か月後には診察で腫瘍は消失しCRと診断されたが, 組織生検は施行されなかった。CR後もS-1を3コース内服したが, 大腿骨頸部骨折により来院困難となり中止となった。CRT終了9か月後に肛門痛を主訴に来院し, 局所再発が確認された。CT検査では遠隔転移を認めなかった。S-1内服を再開したが薬疹のため中止となり, 以後はbest supportive careを行い, CRT終了15か月後に死亡した。遠隔リンパ節への転移を有する膣癌を合併したT1症例は, CRT終了2か月後に肛門癌が消失しCRと診断され, 以後は膣癌に対して化学療法, RTを行った。肛門癌の再発は認めなかったが, CRT終了30か月後に膣癌のため死亡した。

教室の標準治療を施行した他の4例はいずれもstage IIであった。これらの観察期間中央値は1,123 (298〜1,978) 日で, 1例は肛門扁平上皮癌のCR確認後に発症した咽頭癌の治療中であるが, いずれも無再発生存中である。

III. 考 察

肛門扁平上皮癌に対する治療は1974年にNigroがRTに5-FUとMMCを用いたCRTを報告して以来[6], 直接の比較試験は行われていないが, CRTの予後や局所制御率は手術と同等以上であったこと, 腹会陰式直腸切断術では永久人工肛門の弊害があることから, 現在の欧米ではCRTが標準治療として確立されている[2,7]。本邦においても, 第59回大腸癌研究会のアンケート調査報告によれば1989年までは89.0%の症例で腹会陰式直腸切断術が行われていたが, 1995年以降では49.0%とその比率は減少しており[3], 本邦においても肛門扁平上皮癌に対し

てCRTが施行される頻度は，増加傾向にあると考えられる。

肛門扁平上皮癌は，腫瘍サイズが治療成績や予後に関係することからTNM分類のT分類には腫瘍の最大径が用いられ[4]，米国National Comprehensive Cancer Network（NCCN）は肛門扁平上皮癌に対する，stageに応じた治療指針を示している[2]。自験例は高齢のため，S-1を用いた1例を除く全例でNCCNの治療指針に準じた5-FUとMMCによるCRTを施行し，Grade 3の好中球および血小板の減少を1例に認めたが，RTの中断や化学療法の開始を1週間以上延期することなく全例が安全に治療を完遂することができた。しかしMMCでは，Grade 3以上の血液毒性の出現頻度が約60%と高率であるとされ，5-FUとCDDPの併用療法の開発が行われている[8,9]。しかし，5-FU（1,000 mg/m^2 day 1〜4，day 29〜32）とMMC（10 mg/m^2 day 1, 29）を用いたCRTを標準治療として，5-FUとCDDPによる導入化学療法後に5-FU（1,000 mg/m^2 day 57〜60，day 85〜88）とCDDP（75 mg/m^2 day 57, 85）を用いたCRTとを比較した第Ⅲ相試験（RTOG 9811）では，disease free survival（DFS），overall survival（OS）は両群間に差を認めず，colostomy free survival（CFS）と局所制御率は5-FU＋MMCで有意に高率であったこと，Grade 3以上の血液毒性は5-FU＋MMCで有意に高率であったものの，非血液毒性には両群間に差を認めなかったことから，5-FUとMMCを用いたCRTが現時点では標準治療と結論され[10]，5-FU＋CDDPは再発後の治療として位置付けられている[2]。今後，現在進行中のCDDPを組み込んだ第Ⅲ相比較試験（RTOG 98-11，UKCCCR ACT-2，EORTC-22011）によって，肛門扁平上皮癌の治療におけるCDDPの位置付けが明らかとされることが期待される。

さらに英国ではRT（50.4 Gy/28 Fr）にcapecitabine（RT照射日に1,650 mg/m^{2}内服）とMMC（12 mg/m^{2}をday 1に静注）を併用した第Ⅱ相試験EXTRA trialが行われ，今後第Ⅲ相試験が計画される見込みである[11]。一方，本邦においても日本臨床腫瘍研究グループ（JCOG）にて肛門管扁平上皮癌に対してS-1とMMCを用いたCRTの第Ⅱ相試験が行われている[12]。S-1を使用した自験例は十分なdoseを投与できなかったにもかかわらず，T3腫瘍が診察上消失するまでになった。局所再発を認めたものの，患者のquality of life（QOL）に配慮した経口剤による化学療法の開発を期待させる結果であった。

われわれの経験から，肛門扁平上皮癌に対する5-FUとMMCを用いたCRTは安全に施行が可能で，根治が期待される治療法と考えられた。しかし，今後も使用する化学療法，投与量や投与方法，RTの照射量や方法など，患者のQOLを考慮した，より適切な治療法の研究，開発が望まれる。

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Microsomal Epoxide Hydrolase Polymorphisms, Cigarette Smoking, and Risk of Colorectal Cancer: The Fukuoka Colorectal Cancer Study

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Microsomal epoxide hydrolase (EPHX1) plays an important role in the activation and detoxification of polycyclic aromatic hydrocarbons, carcinogens found in cigarette smoke. Polymorphisms in exon 3 (Y113H) and exon 4 (H139R) of the *EPHX1* have been associated with enzyme activity. We investigated the risk of colorectal cancer in relation to the *EPHX1* Y113H and H139R polymorphisms and assessed effect modifications of cigarette smoking and the other covariates. The interaction between the *EPHX1* polymorphisms and selected genetic polymorphisms was also examined. We used data from Fukuoka Colorectal Cancer Study, a community-based case–control study, including 685 cases and 778 controls. In-person interviews were conducted to assess lifestyle factors. The *EPHX1* Y113H and H139R polymorphisms were determined by the TaqMan assay and the polymerase chain reaction-restriction fragment length polymorphism, respectively. Neither of the two polymorphisms nor the imputed EPHX1 phenotype was associated with colorectal cancer risk. Cigarette smoking and alcohol intake showed no effect modification on the association with the *EPHX1* polymorphisms or the imputed EPHX1 phenotype. Increased risks of colorectal cancer associated with the 113Y allele and imputed EPHX1 phenotype were observed among individuals with high body mass index (BMI; $\geq$25.0 kg/m^2), but not among those with low BMI (<25.0 kg/m^2). The risk decreased with an increasing number of the 139R allele in the null genotypes of *GSTM1/GSTT1*. It is unlikely that the *EPHX1* polymorphisms play an important role in colorectal carcinogenesis. The observed interactions of the *EPHX1* polymorphisms with BMI and the *GSTM1/GSTT1* genotypes warrant further investigation.

Key words: microsomal epoxide hydrolase; polymorphism; cigarette smoking; colorectal cancer

INTRODUCTION

Colorectal cancer accounts for 10% of all cancers and is the third most common cancer in the world [1]. In Japan, the temporal trend showed a marked increase in the incidence of and mortality from colorectal cancer until 1990s [2], and the rates are currently among the highest in the world [1]. Risk for colorectal cancer is influenced by both environmental and genetic factors [3]. Several lifestyle factors such as physical inactivity, alcohol use, and high intake of red meat have been implicated in increased risk of colorectal cancer [4]. It has been a matter of controversy whether smoking is related to increased risk of colorectal cancer [5]. Smoking is consistently related to increased risk of colorectal adenomas [6], and a recent meta-analysis reported a small increase in the risk of colorectal cancer associated with long-term smoking although the findings are rather

Abbreviations: EPHX1, microsomal epoxide hydrolase 1; BMI, body mass index; GST, gluthathione *S*-transferase; OR, odds ratio; CI, confidence interval; CYP, cytochrome P-450; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

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disparate [7]. While descriptive features of lung and colorectal cancers are not supportive of a causal role for smoking in colorectal carcinogenesis [8], it is possible that smoking may confer increased risk of colorectal cancer in genetically susceptible individuals in terms of the metabolism of carcinogens in tobacco smoke [9].

Microsomal epoxide hydrolase (EPHX1) is an enzyme involved in the metabolism of reactive epoxides including polycyclic aromatic hydrocarbons, carcinogens found in cigarette smoke [10]. The EPHX1 converts benzo(a)pyrene 7,8 epoxide to the less reactive and more water-soluble dihydrodiol, benzo[a]pyrene 7,8 diol [10]. Although this reaction is generally considered as a detoxification reaction, the less reactive dihydrodiol can be further activated into a highly reactive benzo(a)pyrene 7,8 dihydrodiol 9,10 epoxide [11]. Two functional polymorphisms are known in the *EPHX1* gene; one is the Y113H in exon 3 (rs 1051740), and the other is the H139R in exon 4 (rs 2234922) [12]. In vitro, the *EPHX1* 113H allele is associated with a 40% decrease in enzyme activity, and the 139R allele has an approximately 25% higher activity [12]. Individuals homogyzous or heterozygous for the 113H were shown to have decreased risks of lung cancer [13–15] and upper aerodigestive cancer [16]. Furthermore, high-activity phenotype imputed from the combined genotypes of the Y113H and H139R was associated with increased risks for cancers of the lung [13] and upper aerodigestive tract [16] among those with a high exposure to cigarette smoking. These findings suggest that the *EPHX1* polymorphisms may play a role in the development of tobacco-related cancers. The 113H allele was associated with an increased risk of bladder cancer [17], however.

Several studies have addressed the association of the *EPHX1* polymorphisms with colorectal cancer [18–23] and adenomas [23–28], reporting inconsistent findings. Individuals with the 113HH genotype had an increased risk of colorectal cancer in the earliest study [18] but a decreased risk in the subsequent study [19]. The other studies showed no measurable association of Y113H, H139R, or the imputed phenotype activity with colorectal cancer risk [20–23]. On the other hand, high-activity phenotype was associated with an increased risk of colorectal adenomas among smokers [24,25], whereas individuals homozygous for the 113H allele and those with the composite genotype representing very slow activity showed an increased risk of colorectal adenomas when they had a high exposure to smoking [28]. In the present study, we examined the risk of colorectal cancer in relation to the *EPHX1* Y113H and H139R polymorphisms and assessed the interaction between these polymorphisms and cigarette smoking in the Fukuoka Colorectal Cancer Study, a community-based case–control study in Japan. We also explored the effect modifications of alcohol intake and body mass index (BMI) and the interactions between the *EPHX1* polymorphisms and other genetic polymorphisms of the enzymes involved in tobacco carcinogens.

MATERIALS AND METHODS

Methodological issues of the survey in the Fukuoka Colorectal Cancer Study have been described previously [29]. The study was approved by the ethics committee of the Kyushu University Faculty of Medical Sciences and the collaborating hospitals except two; in the two hospitals, ethics committee was not available at the time of the survey, and the survey was done with an approval of each hospital director.

Subjects

Both cases and controls were residents of Fukuoka city or three adjacent areas. Cases consisted of consecutive patients with histologically confirmed incident colorectal cancer who were admitted to the two university hospitals and six affiliated hospitals for surgical treatment during the period of September 2000 to December 2003. Eligible cases were those aged 20–74 yr at the time of diagnosis and lived in the study area. They also had to be mentally competent to complete the interview. Exclusion criteria were patients who had history of partial or total removal of the colorectum, familial adenomatous polyposis, or inflammatory bowel disease. Of the 1,053 eligible cases, a total of 840 (80%) participated in the interview and 685 gave an informed consent for genotyping.

Controls were frequency matched with cases on sex and 10-yr age class using the same inclusion criteria as for the cases except they did not have a prior diagnosis of colorectal cancer. Exclusion criteria were the same as those for the cases. A total of 1,500 subjects were selected by a two-stage random sampling using residential registry and were invited to participate in the study by mail. Among them, 1,382 were found to be eligible; 833 (60%) participated in the survey, and 778 gave an informed consent for genotyping.

Data Collection

Lifestyle factors were ascertained by in-person interview using a uniform questionnaire. Cases were interviewed in the respective hospitals while controls were interviewed in the public community centers or collaborating clinics. The index date was defined as the date of the onset of symptoms or screening leading to the diagnosis for the cases and the date of interview for controls. BMI (kg/m^2) 10 yr earlier, which was estimated by reported height and weight, was used because the current body mass index was unrelated to colorectal cancer risk [30].

Body weight 10 yr earlier was not available for 2 cases and 10 controls and was substituted with the current body weight. Years of smoking and numbers of cigarettes smoked per day were ascertained for each decade of age if the subjects had ever smoked cigarettes daily for 1 yr or longer. Cigarette-yr until the beginning of the previous decade of age was determined by multiplying the number of cigarettes smoked per day by the years of smoking, and classified into 0, 1–399, 400–799, and ≥800 cigarette-yr. Information on alcohol consumption, type of job and non-occupational physical activity at the time of 5 yr prior to the index date was ascertained. Non-occupational physical activity was expressed as a sum of metabolic equivalents (MET) multiplied by hours of weekly participation in each activity [30].

Genotyping

DNA was extracted from the buffy coat by using a commercial kit (Qiagen GmbH, Hilden, Germany). The following genotyping procedures used 1 μl template DNA with a concentration of 10 ng/μl. Genotyping of the *EPHX1* Y113H polymorphism was carried out by the TaqMan assay (assay ID C_14938_30; Applied Biosystems, Inc., Foster City, CA), using the Stratagene Mx3000P Real-Time QPCR system (Agilent Technologies, Inc., Santa Clara, CA). The *EPHX1* H139R polymorphism was determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described elsewhere [31], using primers 5′-GGTGCCAGAGCCTGACCGTGC-3′ (sense) and 5′-ATGGAACCTCTAGCAGCCCCGTACC-3′ (anti-sense). The PCR product of 319 bp was digested with *Rsa*I, resulting in fragments of 297 and 22 bp for the 139H allele and fragments of 177, 122, and 22 bp for the 139R allele. The digestion products were separated on a 3% agarose gel (NuSieve, Lonza, Rockland, ME).

Statistical Methods

The EPHX1 activity phenotype was imputed on the basis of the number of putative high-activity alleles (113Y and 139R) in the combined genotype [13]. Associations of the *EPHX1* genotypes with colorectal cancer risk were examined in terms of odds ratio (OR) and 95% confidence interval (CI), which were obtained from logistic regression analysis. Statistical adjustment was made for 5-yr age class (starting with the lowest class of <50 yr), sex, residence area (Fukuoka City or the adjacent areas), and smoking (0, 1–399, 400–799, or ≥800 cigarettes-yr). The results did not change with additional adjustment for BMI 10 yr ago (<22.5, 22.5–24.9, 25.0–27.4, or ≥27.5 kg/m^2), alcohol intake (0, 0.1–0.9, 1.0–1.9, or ≥2.0 units/day), type of job (sedentary, moderate, or hard), non-occupational physical activity (0, 1–15.9, or ≥16 MET-h/wk), and parental history of colorectal cancer. Thus, we presented the ORs with adjustment for age, sex, residence area, and smoking.

Trend of the association was assessed with scores 0, 1, and 2 assigned to the three genotype categories. Effect modifications of smoking and the other covariates were tested by the Wald statistic for a product term of the ordinal variable for genotype and a dichotomous variable for smoking (<400 and ≥400 cigarette-yr) [32], alcohol intake (<2.0 and ≥2.0 unit) [33], and BMI (<25.0 and ≥25.0 kg/m^2) [30] with reference to the previous results. Previously, we reported the associations with *Cytochrome P450 (CYP) 1A1*, *Gluthathione S-transferase (GST) M1*, and *GSTT1* polymorphisms in relation to colorectal cancer risk in the same study subjects [34]. Since the EPHX1 is in the interplay with the CYP1A1 and GST in the metabolism of tobacco-related carcinogens [19], interactions between the *EPHX1* polymorphisms and these other polymorphisms (*CYP1A1*2A*, *CYP1A1*2C*, and the combination of the *GSTM1* and *GSTT1* genotypes) were also explored. The Hardy–Weinberg equilibrium was tested using Pearson's χ^2-test with 1 degree of freedom. A two-sided *P*-value <0.05 was considered as statistically significant. Statistical analyses were calculated using SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

Characteristics of cases and controls have been previously reported [33]. In brief, the mean age (SD) of the cases and controls were 60.2 (8.7) and 58.6 (10.7) yr, respectively ($P = 0.003$). Males numbered 426 (62%) in the case group and 490 (63%) in the control group. As compared with controls, cases were more likely to be heavy drinkers, had greater BMI 10 yr earlier, and had a higher frequency of family history of colorectal cancer. Cases and controls were not different with respect to residence area, smoking, type of job and non-occupational physical activity.

Genotype distribution of the controls was in Hardy–Weinberg equilibrium for both the *EPHX1* Y113H ($P = 0.35$) and H139R ($P = 0.41$). Frequencies of the *EPHX1* 113H allele were 0.42 in cases and 0.44 in controls, and frequencies of the *EPHX1* 139R allele were 0.16 in cases and 0.18 in controls. As compared with the *EPHX1* 113YY genotype, the *EPHX1* 113HH genotype was associated with a slightly decreased risk of colorectal cancer. The *EPHX1* 139R allele tended to be related to a decreased risk. These decreases in risk were far from the statistical significance, however. The imputed EPHX1 phenotype activity was unrelated to colorectal cancer (Table 1). Sex-specific analyses showed no difference in the association with the *EPHX1* Y113H, H139R polymorphisms, and the imputed EPHX1 phenotype activity between men and women ($P = 0.94$, 0.82, and 0.93, respectively). The associations did not differ in two age groups of <50 and ≥50 yr ($P = 0.29$ for Y113H, 0.70 for H139R, and 0.37 for the imputed phenotype). Furthermore, we