

Incidence of gastrointestinal toxicity during estramustine phosphate therapy for prostate cancer is associated with the single-nucleotide polymorphisms in the cytochrome P450 1A1 (CYP1A1) gene

Research Article

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Abbreviations: confidence interval, (CI); Cytochrome P450, (CYP); Estramustine phosphate, (EMP); estramustine, (EaM); estromustine, (EoM); Gastrointestinal toxicity, (GIT); intervening sequence, (IVS); luteinizing-hormone releasing-hormone agonist, (LH-RHa); odds ratio, (OR); polymerase chain reaction, (PCR); single nucleotide polymorphisms, (SNPs)

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Summary

Gastrointestinal toxicity (GIT) is observed frequently during estramustine phosphate (EMP) therapy in prostate cancer patients. This adverse effect often deteriorates the patients' compliance and quality of life, which results in drug discontinuation. The CYP1A1 gene is polymorphic and involves in the metabolism of EMP. Polymorphisms of the CYP1A1 gene might have a role to modulate the metabolism of EMP and convert patients' ability to comply with the drug toxicities. We performed genotyping of the CYP1A1 gene to reveal interindividual difference of GIT associated with EMP therapy. The study enrolled 126 patients with untreated advanced prostate cancer. Low-dose of EMP was administered orally. Genotyping of m1, m2 and IVS1-728 polymorphisms of the CYP1A1 gene was

performed by PCR-based direct sequencing method. In the multivariate analysis, GIT risk was increased significantly in the major allele homozygous genotypes of m1 and IVS1-728 SNPs compared with the heterozygous and minor allele homozygous genotypes (T/T genotype of m1, odds ratio (OR), 2.69; 95% CI, 1.24 to 5.96; $P = 0.01$; and G/G genotype of IVS1-728, OR, 3.31; 95% CI, 1.52 to 7.47; $P < 0.01$). Haplotype study showed that the risk of GIT was enhanced approximately 12 times higher when 'T' nucleotide in m1, 'A' nucleotide in m2 and 'G' nucleotide in IVS1-728 locus are present in a same allele (OR, 11.86; 95% CI, 3.61 to 39.02; $P < 0.01$) compared with the other allelic combinations. This study demonstrated that m1, m2 and IVS1-728 polymorphisms in the CYP1A1 gene were significantly associated with GIT during EMP therapy in prostate cancer patients. Genotyping of these polymorphisms prior to commence the EMP therapy will be a useful method to select the patients with a risk of GIT and to improve patients' compliance and quality of life.

I. Introduction

Estramustine phosphate (EMP) is a chemoendocrine agent that was applied for the treatment of prostate cancer in the 1970s. Nowadays, usually it is used for the treatment of hormone refractory prostate cancer. Recently, synergy between EMP and other antimicrotubule agents (i.e. paclitaxel and docetaxel) in the treatment of androgen-independent prostate cancer has been assessed and the clinical efficacy of EMP has been re-evaluated (Vaishampayan et al, 2002; Oudard, 2005).

Gastrointestinal toxicity (GIT) is a frequent adverse effect during EMP therapy in prostate cancer patients. GIT includes anorexia, heartburn, nausea and vomiting. Recently, we conducted a clinical trial of low-dose EMP monotherapy for previously untreated prostate cancer. The object is to achieve the goal of lowering the incidence of adverse effects without compromising the therapeutic efficacy. We found 93.4% of prostate specific antigen (PSA) response rate (Kitamura, 2002), which was comparable with that of conventional dose EMP therapy. However, incidence of the toxicity remained rather high as 39.5% and discontinuation rate was 32.1%. Individual susceptibility to the adverse effects perhaps linked with the polymorphisms of the enzymes encoding genes that are related to the metabolism of this drug.

Figure 1 is a schematic diagram showing the metabolic pathway of EMP. EMP consists of 17 β -estradiol bound to nor-nitrogen mustard. After ingestion, EMP undergoes rapid dephosphorylation to yield estramustine (EaM), and EaM is oxidized by 17 β -hydroxysteroid dehydrogenases to estromustine (EoM). EaM and EoM then yield 17 β -estradiol and estrone, respectively, by hydrolysis (Gunnarsson et al, 1984).

Furthermore, 17 β -estradiol is hydroxylated by Cytochrome P450 (CYP) 1A1, 1A2 and 3A4 to yield 2-hydroxyestradiol (Lakhani et al, 2003). 2-hydroxyestradiol can compete with dopamine receptor effectively (Schaeffer et al, 1979). It was also reported that 2-hydroxyestradiol behaves like an antagonist for dopaminergic receptors in striatum and pituitary (Paden et al, 1982).

The m1 (3801T>C) and intervening sequence (IVS) 1-728 (G>A) are intronic single nucleotide polymorphisms (SNPs), and m2 (2455A>G) is an exonic SNP of the CYP1A1 gene. The minor genotypes of m1 and m2 polymorphisms have been reported to increase its enzymatic activity (Cosma et al, 1993; Crofs et al, 1994), and reflect the mass of catechol estrogen transformation from 17 β -estradiol and estrone. Thus, the SNPs of the

CYP1A1 gene might play a role in modulating the metabolism of EMP and act as one of the determining factors for the inter-individual variations in adverse effects of EMP therapy. To find out the link between genotypes encoding the enzymes of EMP metabolism and its adverse effects, we evaluated m1, m2, and IVS1-728 polymorphisms in the CYP1A1 gene among the prostate cancer patients treated with EMP.

II. Materials and methods

A. Study design and treatment plan

A total of 126 patients with untreated advanced prostate cancer were enrolled in this study (**Table 1**). Age range was 48 to 89 years (mean 72.5 years). Patients with significant active concurrent medical illness, other malignancy, or cardiovascular diseases were excluded from this study. The Ethics Committee of the University of Tokyo approved this study and prior to study written informed consent was obtained from each patient. The treatment regimens were oral EMP alone (280 mg/day) for 42 patients, oral EMP (280 mg/day) plus luteinizing-hormone releasing-hormone agonist (LH-RHa) or surgical castration for 13 patients, and oral EMP (140 mg/day) plus LH-RHa for 71 patients. Physical condition of the patients and incidence of the adverse effects were assessed monthly on the basis of the National Cancer Institute-Common Toxicity Criteria (Version 2.0, Jan. 30, 1998). According to their medical reports, 42 of 126 (33.3%) patients suffered from GIT during the low-dose EMP therapy. Then, we compared the genotypes of SNPs in the CYP1A1 gene among the patients with or without GIT.

B. Genotyping assay

Genotyping was performed by polymerase chain reaction (PCR) based-direct sequencing method. Genomic DNA was extracted from the peripheral blood lymphocytes by standard method. To use it in PCR solution, concentration of genomic DNA was adjusted to 100 ng/ μ L. Primers used for DNA amplification are forward 5'-CAG TGA AGA GGT GTA GCC GCT-3' and reverse 5'-TAG GAG TCT TGT CTC ATG CCT-3' for m1; forward 5'-AGT GGC ACG CTG AAT TCC A-3' and reverse 5'-CCC CTG ATG GTG CTA TCG AC-3' for m2; forward 5'-TGT TCT CAG GGG AAT TAG GG-3' and reverse 5'-AAG CAA TGT GGT TTG GGA AG-3' for IVS1-728. Melting temperature were 60°C for m1 and IVS1-728, 58°C for m2. Cyclic thermal conditions were 95°C for 10 minutes for one cycle; 94°C for 30 seconds, melting temperature for each set of primers, for 30 seconds, 72°C for 3 minutes for 37 cycles; followed by a cycle of 72°C for 10 minutes. The amplification reactions of each SNP were performed in 50 μ L solution containing 100 ng of genomic DNA, 5 μ L of 10xPCR Gold Buffer, 1.5 mmol MgCl₂ solution, 0.2

mmol dNTPs, 1.25 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Branchburg, NJ, USA), and 0.5 μ M of each specific primer (synthesized by Fasmac, Atsugi, Kanagawa, Japan). GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) was used to perform PCR. All PCR amplicons were purified with the Montage PCR μ 96 Plate (Millipore Corporation, Bedford, MA, USA) to remove deoxynucleotide triphosphates and excess primers. All sequencing reactions were performed by dye terminator chemistry (ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit; Applied Biosystems, Warrington, UK) with each sequencing primer, and the products were purified with the MultiScreen filter plates (Millipore Corporation, Bedford, MA, USA) with Sephadex G-50 Superfine (Amersham Biosciences, Uppsala, Sweden). Purified samples were applied to an ABI Prism 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA), and sites of polymorphisms were identified with Sequencher software (version 4.1.2; Gene Codes Corporation, Ann Arbor, MI, USA).

C. Statistical analysis

The allele frequency of each SNP was estimated by direct count and assessed for deviation from the Hardy-Weinberg equilibrium by using χ^2 tests. Univariate and multivariate analyses were conducted by nominal logistic regression analysis. In the multivariate analysis, OR, 95% CI and *P* value were obtained after adjustment for age, baseline PSA level, performance status, clinical stage, Gleason score and dose of EMP. EH program (version 1.20) was used for haplotype analyses. These statistical analyses were conducted by JMP software, version 5.1.2 (SAS, Cary, NC) and the *P* value was considered as significant when it was less than 0.05.

Table 1. Patient characteristics.

Characteristics	Number
Age (mean \pm SD)	72.5 \pm 8.8 years
Baseline PSA (mean \pm SE)	418.3 \pm 106.7 ng/mL
Performance status	
0	93
1	26
2	7
Clinical stage	
C	60
D	66
Gleason score	
2 to 7	71
8 to 10	55
Dose of EMP	
140 mg/day	71
280 mg/day	55

SD, standard deviation; SE, standard error; PSA, prostate specific antigen.

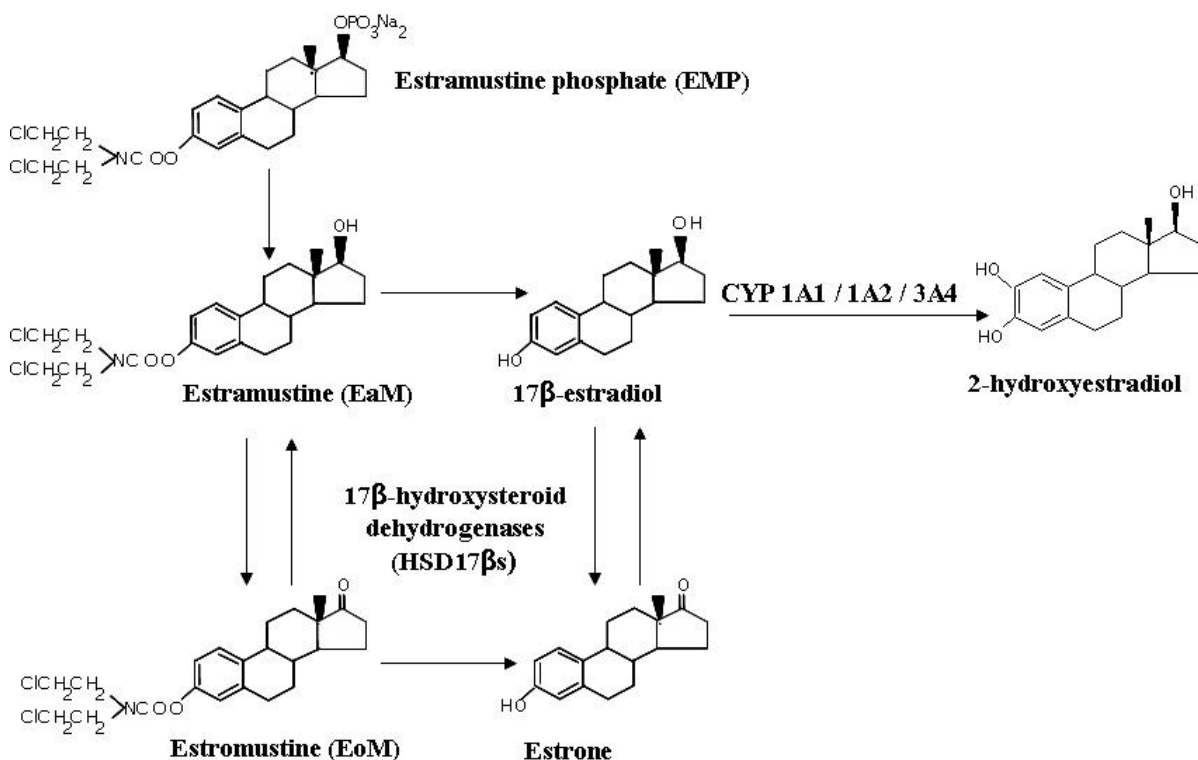


Figure 1. Metabolic pathway of estramustine phosphate (EMP). EMP is dephosphorylated to yield estramustine (EaM). EaM is oxidized by 17 β -hydroxysteroid dehydrogenases (HSD17Bs) to estromustine (EoM). EaM and EoM then yield 17 β -estradiol and estrone, respectively, by hydrolysis. Furthermore, 17 β -estradiol is hydroxylated by Cytochrome P450 (CYP) 1A1, 1A2 and 3A4 to yield 2-hydroxyestradiol.

III. Results

Of the 126 patients, 42 (33.3%) suffered from GIT during low-dose EMP therapy. Seventy-one patients were administered EMP at a dose of 140 mg/day and 21 (29.6%) of them suffered from GIT. Fifty-five patients were administered EMP at a dose of 280 mg/day and 21 (38.2%) of them suffered from GIT. Incidence of GIT was more frequent in the group of EMP 280 mg/day, however, it was not statistically significant ($\chi^2 = 1.03, P = 0.31$).

Table 2 shows grade and symptoms of GIT. Approximately 70% of total GIT were grade 1 and the most common GIT was anorexia (45.2%). One patient developed hematemesis associated with grade 3 gastric ulcer.

We performed genotyping assay for 3 SNPs in the CYP1A1 gene and frequency of each genotype is shown in **Table 3** and **4**. The distribution of genotypes of these SNPs did not deviate from the Hardy-Weinberg equilibrium. It was observed that the major allele

homozygous genotypes of m1 and IVS1-728 are frequent among the patients suffered from GIT. The associations between genotypes of m1, m2, and IVS1-728 polymorphisms with GIT are shown in **Table 5**. The major alleles homozygous genotypes of m1 and IVS1-728 polymorphisms are significantly frequent among the patients suffered from GIT. Univariate analysis shows that the frequency of T/T genotype of m1 and G/G genotype of IVS1-728 are significantly associated with GIT (T/T genotype of m1, OR 2.78; 95% CI, 1.31 to 6.07; $P < 0.01$; and G/G genotype of IVS1-728, OR 3.41; 95% CI, 1.59 to 7.56; $P < 0.01$). Multivariate analysis demonstrated an independent significant relationship between m1, IVS1-728 polymorphisms and GIT (T/T genotype of m1, OR 2.69; 95% CI, 1.24 to 5.96; $P = 0.01$; and G/G genotype of IVS1-728, OR 3.31; 95% CI, 1.52 to 7.47; $P < 0.01$).

Table 2. GIT observed during EMP therapy (n = 42).

	GIT grade of the NCI-CTC			
	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Total n (%)
Anorexia	14 (33.3)	5 (11.9)	0	19 (45.2)
Heartburn	11 (26.2)	3 (7.1)	0	14 (33.3)
Nausea and Vomiting	5 (11.9)	3 (7.1)	0	8 (19.0)
Gastric ulcer	0	0	1 (2.4)	1 (2.4)
Total, n (%)	30 (71.4)	11 (26.2)	1 (2.4)	42 (100)

GIT, gastrointestinal toxicity; NCI-CTC, National Cancer Institute-Common Toxicity Criteria.

Table 3. Allele frequency of m1, m2, and IVS1-728 polymorphisms with Hardy-Weinberg equilibrium test.

dbSNP ID	rs4646903	rs1048943	rs4646421
Trivial name	m1	m2	-
Position	3801T>C	2455A>G	IVS1-728G>A
Allele frequency	Major allele	T: 0.66	A: 0.79
	Minor allele	C: 0.34	G: 0.21
HWE ²	0.44	2.05	0.61
P value	NS	NS	NS

-, not applicable; HWE, Hardy-Weinberg equilibrium; NS, not significant.

Table 4. Distribution of genotypes in m1, m2, and IVS1-728 loci with or without GIT.

Genotypes	Locus							
	m1		m2		IVS1-728			
	GIT (+), n=42	GIT (-), n=84	Genotypes	GIT (+), n=42	GIT (-), n=84	Genotypes	GIT (+), n=42	GIT (-), n=84
C/C	4 (9.5)*	12(14.3)	G/G	1 (2.4)	7(8.3)	A/A	4 (9.5)	13(15.5)
T/C	12 (28.6)	41 (48.8)	A/G	10 (23.8)	26 (31.0)	G/A	11 (26.2)	42(50.0)
T/T	26 (61.9)	31 (36.9)	A/A	31 (73.8)	51 (60.7)	G/G	27 (64.3)	29(34.5)

* No. of patients (%), GIT, gastrointestinal toxicity; IVS, intervening sequence.

The results of haplotype analyses are shown in **Table 6**. It is recognized from haplotype analyses that the chance of GIT is over two times higher when 'A' nucleotide in m2 and 'G' nucleotide in IVS1-728 loci are present in a same allele compared with other combinations (OR, 2.33; 95% CI, 1.00 to 5.41; $P = 0.05$). Furthermore, when the patients represent 'T' nucleotide in m1, 'A' nucleotide in m2 and 'G' nucleotide in IVS1-728 locations in a same allele, the risk of GIT was enhanced approximately 12 times higher than other allelic combinations (m1+m2+IVS1-728 = T+A+G, OR, 11.86; 95% CI, 3.61 to 39.01; $P < 0.01$).

IV. Discussion

Withdrawal of chemotherapy due to drug toxicities may allow disease progression and worsen the survival of

the patients. Also additional treatments and measures are required to manage the patients for adverse effects. Therefore, selection of suitable patients prior to commence the therapy might be beneficial for survival of patients as well as medical economy.

GIT is one of the major adverse effects of EMP therapy. About 25.9% patients suffered from GIT during EMP therapy (Kitamura, 2001 and 2002). To overcome this adverse effect is crucial, however, the mechanism of GIT during EMP therapy is not well known.

The allele frequencies of these three polymorphisms are shown in the database of National Cancer Institute (NCI) (<http://snp500cancer.nci.nih.gov/snp.cfm>). Allelic frequencies of m1 and IVS1-728 in our patients are very similar to that of NCI data. However, minor allelic frequency of m2 among the patients appeared higher than

Table 5. Relation between polymorphisms of the CYP1A1 gene and risk of GIT (univariate and multivariate analyses).

Factors	Univariate			Multivariate		
	OR	95% CI	P value	OR†	95% CI†	P value
m1						
T/C & C/C	1.0*			1.0*		
T/T	2.78	1.31 to 6.07	< 0.01	2.69	1.24 to 5.96	0.01
m2						
A/G & G/G	1.0*					
A/A	1.82	0.82 to 4.25	NS	1.83	0.81 to 4.39	NS
IVS1-728						
G/A & A/A	1.0*			1.0*		
G/G	3.41	1.59 to 7.56	< 0.01	3.314	1.52 to 7.47	< 0.01
Age						
< 73	1.0*			1.0*		
73	0.68	0.32 to 1.43	NS	0.80	0.37 to 1.74	NS
Baseline PSA level						
< 418 ng/mL	1.0*			1.0*		
418 ng/mL	1.09	0.38 to 2.92	NS	1.13	0.35 to 3.46	NS
Performance status						
0 and 1	1.0*			1.0*		
2	0.32	0.02 to 1.94	NS	0.25	0.01 to 1.69	NS
Clinical stage						
C	1.0*			1.0*		
D	0.87	0.41 to 1.82	NS	0.71	0.30 to 1.64	NS
Gleason score						
2 to 7	1.0*			1.0*		
8 to 10	1.27	0.60 to 2.69	NS	1.35	0.59 to 3.09	NS
Dose of EMP						
140 mg/day	1.0*			1.0*		
280 mg/day	1.47	0.70 to 3.11	NS	1.72	0.78 to 3.81	NS

IVS, intervening sequence; EMP, estramustine phosphate; PSA, prostate-specific antigen; OR, odds ratio; CI, confidence interval; NS, not significant.

*Reference

†OR, 95% CI and P value were obtained after adjustment for age, baseline PSA level, performance status, clinical stage, Gleason score, and dose of EMP.

Table 6. Results of haplotype analyses between m1, m2, and IVS1-728 polymorphisms.

Allelic combination	Number of patients		OR (95% CI)	P value
	GIT (+)	GIT (-)		
m1+m2 = T+A	32.00	50.91	2.08 (0.90 to 4.79)	NS
others	10.00	33.09		
m1+IVS1-728 = T+G	32.00	50.00	2.18 (0.95 to 5.01)	NS
others	10.00	34.00		
m2+IVS1-728 = A+G	32.50	50.00	2.33 (1.00 to 5.41)	0.05
others	9.50	34.00		
m1+m2+IVS1-728 = T+A+G	38.62	41.18	11.86 (3.61 to 39.02)	< 0.01
others	3.39	42.82		

IVS, intervening sequence; GIT, gastrointestinal toxicity; OR, odds ratio; CI, confidence interval; NS, not significant.

that of NCI data (our data, A: G = 0.79: 0.21; NCI data, A: G=0.88: 0.12). Perhaps this variation is due to racial differences and the factors related to the disease. Further studies are needed to clarify the cause of this variation.

Our data demonstrated an association between genetic polymorphisms of m1, m2 and IVS1-728 in the CYP1A1 gene and GIT during the EMP therapy. Therefore, genotyping of these polymorphisms prior to EMP therapy will be useful to estimate the risk of GIT. At the present, we could not compare the serum 2-hydroxyestradiol levels on the basis of genotypes. However, the mechanisms of emesis and comparative study of serum 2-hydroxyestradiol levels in different genotypes of these three SNPs during EMP therapy are remaining for future study. Our results are novel and the association was statistically significant, however, much larger population is needed to confirm our data. Because, in the haplotype analyses, one allelic subgroup had less than five subjects.

From the results reported herein, genotyping of these three SNPs could be a useful tool to find out the risk group of patients for GIT prior to commence the EMP therapy in prostate cancer. Finally, using a tailor-made medication by detecting the risk group, the drug compliance of EMP could be increased markedly.

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