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The Effect of CYP Polymorphism on Resistance against Praziquantel in *Clonorchis Sinensis*-infected Patients

Chung Hyeon Kim[1,2], Min Ho Choi[2], Jong Il Chae[2], Eun Hee Shin[2] & Sung Tae Hong[2]

[1]Department of Internal Medicine, Healthcare System Gangnam Center, Seoul National University Hospital, 38F, 39F Gangnam Finance Center, #737 Yeoksam-dong, Gangnam-gu, Seoul 135-984, Korea
[2]Department of Parasitology, Seoul National University, College of Medicine, Yeongeon-dong, Jongro-gu, Seoul 110-744, Korea
Correspondence and requests for materials should be addressed to S.T. Hong (HST@snu.ac.kr)

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Abstract

Currently praziquantel is used for treatment of not only clonorchiasis but also other trematodes and cestodes. But cure rate of praziquantel is just 60-80% for most trematodes. It needs for the treatment -failed patients to have more drugs. The cause of failure of treatment is not studied. We just know that the blood level of praziquantel is severely different among the people. We guess that this factor may influence the results of treatment. In an endemic area of human clonorchiasis in Heilongjiang Providence, China, 78 subjects were selected for the study. Three doses of 25 mg/kg (total 75 mg/kg) of praziquantel were administered to 78 clonorchiasis patients. After three weeks of treatment, stool examination was undertaken once again to confirm the cured and uncured subjects. To analyze SNP (single nucleotide polymorphism) of CYP3A5 PS2-1, CYP3A5 PS2-2, and CYP3A5*6, PCR method was done with specifically designed primers. The mutation rates of all sites were not significant statistically. The number of subjects was too small, so we need more subjects and other delivery proteins of bile ducts (ex. MRP etc.) were also considered for effects of praziquantel. We analyzed, for the first time, the entire CYP3A5 gene in a French population, using a polymerase chain reaction- single strand conformational polymorphism (PCR-SSCP) strategy.

Keywords: Praziquantel, Clonorchis sinensis, SNP, CYP

Currently praziquantel is used for treatment of not only clonorchiasis but also other trematodes and cestodes. Especially it is important of the treatment of schstosomiasis that 200 millions of persons are considered to be infested in worldwide. But cure rate of praziquantel is just 60-80% for most trematodes[1]. It needs for the treatment-failed patients to have more drugs. The cause of failure of treatment was not studied. We just know that the blood level of praziquantel is severely different among the people[1]. We guess that this factor may influence the results of treatment. The personal difference with blood level of praziquantel may be the result of absorption, metabolism, and excretion. The killing effect comes from metabolites of praziquantel in bile acid, so we need the process of metabolism of praziquantel and it was important role of this problem.

The clonorchiasis is now prevalent in South Korea, but the cure rate is 83% at most[2]. Clonorchiasis need more drugs than intestinal trematodes and schistosomiasis. It is caused by that intestinal trematodes reside in lumen of intestine so praziquantel acts directly on them, and schistosomas live in the blood, so most metabolites of praziquantel effectively act. But clonorchis exists in bile ducts so a part of the metabolites in blood act on clonorchis[3]. We suggest that the enzymes related with metabolism of praziquantel must exist, and they should have a different between the cured and the failed patients.

The related enzyme of this process is CYP450. It has several families. Of them, CYP3 is related with drug metabolism[4]. So CYP3 subfamily was considered as most important enzyme to metabolite the drug. Of the CYP3 subfamily, CYP3A5 is well known as the enzyme for metabolism of many drugs including praziquantel. The full sequence of CYP3A5 is already known. We examined the SNP of 10 gene sites of CYP3A5 and wanted to reveal the difference of the rate of mutation between the two groups.

There was no difference between the cured and uncured groups in general characteristics (Table 1). We tried to recruit more subjects. But many persons were lost during the term of follow up. Western blots did not show other strands on the field (results not shown). The bands of mutant protein were not shown. We thought that it owed to small concentration of proteins or the wrong condition of PCR test. But several reexaminations were not successful. The results were not changed. The mutation rates of all sites were not significant statistically between the cured

Table 1. General characteristics of *Clonorchis sinensis*-infected patients.

	Praziquantel -cured groups (n=51)	Praziquantel -uncured groups (n=27)	Significance
Age	40.1±10.5	45.0±11.4	0.067
Sex, n (%)			
Male	28 (54.9)	14 (51.9)	0.492
Female	23 (45.1)	13 (48.1)	
EPG counts, n			
Pre-treatment	3009.9±4458.1	4232.0±6039.5	0.313
Post-treatment	0	280.9±243.6	$P<0.001$

Table 2. Frequency of CYP3A5 polymorphism in *Clonorchis sinensis*-infected patients.

	Praziquantel -cured groups (n=51)	Praziquantel -uncured groups (n=27)	Significance
CYP3A5-7	13 (25.5%)	6 (22.2%)	P=0.153 χ^2=1.784 df=1
PS2-1	5 (9.8%)	2 (7.4%)	P=0.659 χ^2=0.005 df=1
PS2-2	13 (25.5%)	6 (22.2%)	P=0.419 χ^2=0.238 df=1

and uncured groups (Table 2).

Discussion

The aim of the present study was to find the cause for resistance of praziquantel. We know that CYP3 family is most related with drug metabolism, and it has frequent polymorphisms in human gene[5]. But, consequently the sites that we selected were all not shown the significant meanings. Six gene sites were examined. Three sites of them were reactive in PCR method, but three of them were not. The reason was not clear.

Praziquantel is excreted into bile juice after metabolism, so metabolism process is very important in treatment of *C. sinensis* and *F. hepatica*. Basically praziquantel metabolism process includes hydroxylation and glucuronization. All processes happen in microsome of hepatocyte. By this process, metabolites are changed to hydrophilic materials and excreted into bile juice or urine. Hydroylated praziquantel has low killing potency, and remained potency acts as vermicide. But glucuronization of the drug makes no effect as vermicide[4]. Therefore it is necessary that absorbed praziquantel must be secreted enough into bile juice through hydroxylation for extermination.

CYP450 is enzyme family with heme. It exists mainly in microsome of mitochondria of animals and plants. The function of this enzyme is the biosynthesis of steroid hormone and mediated oxidative metabolism of extrinsic material, especially drugs. It distributes in the membrane of endoplasmic reticulum of hepatocyte, and intestine in major, and partially in other tissues. It consists of 4 families and 30 isoenzymes. CYP3A4, CYP2D6, CYP1A2, CYP2C9, CYP2C19, and CYP2E1 are related with drug metabolism. These have frequent genetic polymorphism[5]. It is not known clearly that any enzyme is important to hydroxylation metabolism of praziquantel, but generally it is known that 3 family of CYP450 is most important in this process[4,6-8].

All genes were not significant statistically. As the incidence of mutation of CYP was considered, the numbers of subjects were too small. We think that more patients and more sites of the gene might be needed to analyze the effect for resistance of praziquantel. In addition to this result, we suggest that the other hepatobiliary transporter proteins like multidrug resistance-associated protein (MRP) should be studied because they have also important role to excretion of metabolites of many drugs.

Methods

Subjects

In an endemic area of human clonorchiasis in Heilongjiang Providence, China, 125 subjects were randomly selected for fecal examination and tested either positive or negative for clonorchiasis. Out of 125 subjects, 78 subjects were diagnosed as positive for clonorchiasis. These 78 subjects were selected for the study.

Analysis of Single Nucleotide Polymorphism (SNP)

Three doses of 25 mg/kg (total 75 mg/kg) of praziquantel were administered to 78 clonorchiasis patients. After three weeks of treatment, stool examination was undertaken once again to confirm the cured and uncured subjects.

Blood were sampled from each individual and kept by absorbing to filter papers for long period. Chelex-100 resin (BioRad, CA) was used to extract DNA from blood absorbed filter papers. To analyze SNP of CYP3A5 PS2-1, CYP3A5 PS2-2, and CYP3A5-6, PCR method was done with specifically designed primers (Table 3). PCR method was employed 35 cycles

Table 3. Primers for CYP1A2 and CYP3A5 gene polymorphisms.

CYP3A5	Sequence	Size (bp)
CYP3A5*6	5′-GCCGAGACGCACCATTACACT-3′ 5′-ATGAGTGGGAAGGGATGGGTG-3′	244
PS2-1	5′-ACAGGCACAGAAACCCACAAG-3′ 5′-ATCGCCACTTGCCTTCTTC-3′	630
PS2-2	5′-CCCTGCTTCGGCTTGTGCA-3′ 5′-CCTCTTCACAGCCTGCTTTATTTGTCA-3′	575

of 94˚C for 30 seconds, 55-60˚C (depending on the primers) for 30 seconds and 72˚C for 1 minute. The sequences of these genes were analyzed by automatic sequence detection system (ABI 7700, AppliedBiosystems, CA, USA) and SNPs were analyzed using DNASTAR (DNASTAR Inc., WI, USA).

Statistical Analysis

All data were statistically analyzed using chi square method SPSS program (v. 11.0: SPSS Institute, Inc., IL).

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