Effect of MDR1 gene polymorphisms on mortality in paraquat intoxicated patients

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Paraquat is a fatal herbicide following acute exposure. Previous studies have suggested that multidrug resistance protein 1 (MDR1) might help remove paraquat from the lungs and the kidney. MDR1 single-nucleotide polymorphisms (SNPs) are involved in the pharmacokinetics of many drugs. The purpose of this study was to determine whether MDR1 SNPs were associated with the mortality in paraquat intoxicated patients. We recruited 109 patients admitted with acute paraquat poisoning. They were genotyped for C1236T, G2677T/A, and C3435T single-nucleotide polymorphisms (SNPs) of MDR1 gene. Their effects on mortality of paraquat intoxicated patients were evaluated. Overall mortality rate was 66.1%. Regarding the C1236T of the MDR1 gene polymorphism, 21 (19.3%) had the wild type MDR1 while 88 (80.7%) had homozygous mutation. Regarding the C3435T MDR1 gene polymorphism, 37 (33.9%) patients had the wild type, 23 (21.1%) had heterozygous mutation, and 49 (45.0%) had homozygous mutation. Regarding the G2677T/A MDR1 gene polymorphism, 38 (34.9%) patients had the wild type, 57 (52.3%) had heterozygous mutation, and 14 (12.8%) had homozygous mutation. None of the individual mutations or combination of mutations (two or three) of MDR1 SNP genotypes altered the mortality rate. The mortality rate was not significantly different among SNP groups of patients with <4.0 μg/mL paraquat. In conclusion, MDR1 SNPs have no effect on the mortality rate of paraquat intoxicated patients.
Genotyping conditions for the MDR1 gene polymorphisms.

| SNP          | Primer sequence | Annealing temperature (°C) | Enzyme | Cleavage products(bp) |
|--------------|----------------|---------------------------|--------|-----------------------|
| C1238T       | F: TATCCGTGTCGTGAATTGCC | 54 | HaeIII | 370 (272, 98/272, 98, 63, 35/272, 63, 35) |
| (rs1128503)  | R: CGCTGACATCAACACCAATG | | | |
| C3435T       | F: GTGTTTCAGCTGCTTGATG | 53 | San3AI | 197 (197/197, 158, 39/158, 39) |
| (rs1045642)  | R: AGAGGCTATGTGTGGCGCTC | | | |
| G2677T       | F: TGCAGGCTATAGGTCAGG | 58 | BanI | 224 (224/224, 198, 26/198, 26) |
| (rs2032582)  | R: TTTAGTTGACTACCTTCCGG | | | |
| G2677A       | F: TGCAGGCTATAGGTCAGG | 58 | BsrI | 220 (220/220, 206, 14/206, 14) |
| (rs2032582A) | R: GTGGTACCTACCTTCAGG | | | |

Table 1.

Three MDR1 SNPs (rs2032582, rs1045642, and rs1128503) were genotyped in PQ intoxicated patients to evaluate the association between ATP-binding cassette sub-family B member 1 gene (ABCB1/MDR1) and PQ intoxication. These SNPs were selected from a previous study and the National Center for Biotechnology Information (NCBI) website (http://www.ensembl.org; www.ncbi.nlm.nih.gov/SNP). DNA was extracted from peripheral blood using a PureHelix Genomic DNA Prep kit (NanoHelix Co., Ltd., Daejeon, Korea) as described previously.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) protocols are summarized in Table 1. Primer sequences and annealing temperatures used for the analysis of each polymorphism are also listed in Table 1. Each reaction consisted of a single denaturation step at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 sec, annealing with appropriate primer pair at annealing temperature for 30 sec, and extension at 72 °C for 30 sec. A final extension step at 72 °C was performed at the end of the PCR program for 10 min. Following PCR amplification, products were digested overnight with corresponding restriction enzymes (Table 1) according to the manufacturer's instructions. The digested products were electrophoresed on 3.0% agarose gels and stained with SYBR-Green (Invitrogen, Carlsbad, CA, USA). All restriction enzymes used in this study were purchased from New England Biolabs (Ipswich, MA, USA). Reproducibility of genotyping was assured by conducting duplicate experiments. Genotype analysis was conducted by blinding the case and control status.
Statistical analysis. Continuous variables are expressed as mean ± standard deviation with or without the median value and range. Categorical variables are shown as frequencies (number of cases and percentages). Differences between groups were detected using chi-square test or Fisher’s exact test for categorical variables. Binary logistic regression analysis was used to identify the risk of mortality according to gene polymorphism. Results of the logistic regression analyses are reported as relative risks or odds ratios with 95% confidence intervals. Statistical analyses were performed using SPSS ver. 14.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance was considered when P-value was less than 0.05.

Results
Baseline characteristics of the study population. A total of 109 patients were included in this study. Baseline laboratory parameters and initial patient information recorded at hospital arrival are summarized in Table 2. Overall mortality rate was 66.1%, similar to the mortalities rates in previous studies. \(^1\,^2\,^3\) Semi-quantitative dithionite tests revealed that 27 patients had grade 1+ (blue color), 22 patients had grade 2+ (tender blue), and 60 patients had grade 3+ (dark blue) paraquat levels. The mean time to death was 4.67 ± 5.11 days. The frequencies of the three MDR1 SNP genotypes (C3435T, C1236T, and G2677T/A) in PQ intoxicated patients are shown in Table 3.

Effect of MDR1 gene polymorphisms on mortality. Regarding the frequencies of C1236T MDR1 gene polymorphism, 21 (19.3%) patients had the wild type, 88 (80.7%) patients had the homozygous mutation, and none had the heterozygous mutation. The frequencies of C1236T MDR1 polymorphism in survivors were not significantly different from those in non-survivors (\(\chi^2 = 3.604, P\)-value = 0.165). Regarding the frequencies of C3435T MDR1 polymorphism, 37 (33.9%) patients had the wild type, 23 (21.1%) patients had heterozygous mutation, and 49 (45.0%) patients had homozygous mutation. The frequency distributions of the C3435T MDR1 SNP were not significantly different between survivors and non-survivors either (\(\chi^2 = 0.004, P\)-value = 0.947). Regarding the frequencies of G2677T/A MDR-1 polymorphism, 38 (34.9%) patients had the wild type, 57 (52.3%) patients had the heterozygous mutation, and 14 (12.8%) patients had homozygous mutation. The frequency distributions of the C2677T/A MDR1 polymorphism were not significantly different between survivors and non-survivors (\(\chi^2 = 1.506, P\)-value = 0.471). Neither heterozygous nor homozygous mutation of the three MDR1 gene polymorphisms had any effect on the mortality of PQ intoxicated patients (Table 3).

Effect of MDR1 gene polymorphism on mortality in patients with <4.0 μg/mL paraquat. Patients with high plasma PQ concentrations were included in this study. We reanalyzed the 67 patients with <4.0 μg/mL PQ concentration. However, none of the mutations in MDR1 gene affected the mortality of these patients (Table 4). The genotype frequencies of MDR1 SNPs were not associated with mortality either.
Synergistic effect of two or three MDR1 SNP genotypes on mortality. We analyzed the frequency distributions of genotypes of two or three MDR1 SNPs between survivors and non-survivors to investigate the synergistic effect of two or three MDR1 SNPs on mortality (Table 5). When the effect of two or three SNPs in different combinations was evaluated among patients with wild or heterozygous mutant (represented as 0) or homozygous mutant genotype (represented as 1), the frequencies of the SNP combinations bearing the mutant genotype were not significantly different between survivors and non-survivors.

Discussion
P-gp is a glycosylated membrane-bound efflux pump protein that removes substrates from the inside to the outside of the cell. Some reports have shown that inducing P-gp can protect cells against PQ-induced toxicity in vivo and in vitro. Silva et al. have demonstrated that inducing P-gp in Caco-2 cells using newly synthesized thiioxanthones can prevent PQ cytotoxicity. Mice treated with dexamethasone display increased MDR1 expression in the lungs associated with decreased PQ accumulation and pneumotoxicity. In addition to the lungs and liver, MDR1 and Mdr1a/1b are also expressed in human and rodent kidneys, respectively. Xia et al. have shown MDR1/Mdr1 participates in the elimination of PQ from the kidneys and protects against subsequent toxicity. These results suggest that P-gp is involved in the PQ intoxication mechanisms.

MDR1 gene polymorphisms have been associated with altered drug absorption, disposition, and toxicity responses. Among MDR1 SNPs, C1236T in exon 12, G2677T/A in exon 21, and C3435T in exon 26 have been investigated extensively. For example, renal transplant recipients with homozygous mutation in G2677T/A require higher tacrolimus dose than recipients without such mutation to receive the same therapeutic effect. The MDR1 C3435T and G2677T/A polymorphisms are risk factors for increased susceptibility to nephrotic syndrome and steroid resistance. It is currently unclear whether MDR1 genetic polymorphisms can affect the pharmacokinetics and toxicities of PQ. However, variations in MDR1 expression between individuals may alter susceptibility to PQ-induced toxicity. This is the first study to investigate the effect of MDR1 SNPs on mortality of PQ intoxicated patients. In this study, the frequency distributions of homozygous and heterozygous mutation for the G2677T/A, C3435T, and C1236T SNPs were not different between non-survivors and survivors of PQ intoxication.

Table 3. Distribution of the MDR1 gene polymorphisms in paraquat intoxicated patients.

| SNP       | Survival N(%) | Non-survival N(%) | OR     | 95% CI       | P-value |
|-----------|---------------|-------------------|--------|--------------|---------|
| C1236T    |               |                   |        |              |         |
| CC        | 7 (18.9%)     | 14 (19.4%)        | 0.967  | 0.335–2.655 | 0.947   |
| TT        | 30 (81.1%)    | 58 (80.6%)        |        |              |         |
| C3435T    |               |                   |        |              |         |
| CC        | 15 (40.5%)    | 22 (30.6%)        |        |              |         |
| CT        | 10 (27%)      | 13 (18.1%)        | 0.866  | 0.309–2.542 | 0.822   |
| TT        | 12 (32.4%)    | 37 (51.4%)        | 2.102  | 0.834–5.299 | 0.155   |
| CT+TT     | 22 (59.4%)    | 50 (69.5%)        | 1.155  | 0.678–3.539 | 0.299   |
| G2677T/A  |               |                   |        |              |         |
| GG        | 15 (40.5%)    | 23 (31.9%)        |        |              |         |
| GT or A   | 19 (51.4%)    | 38 (52.8%)        | 1.304  | 0.556–3.059 | 0.541   |
| TT or A   | 5 (8.1%)      | 11 (15.3%)        | 2.391  | 0.571–10.020| 0.233   |
| GT or A+TT or A | 22 (59.5%) | 49 (68.1%) | 1.453 | 0.638–3.306 | 0.374   |

Table 4. Distribution of the MDR-1 gene polymorphisms in patients with <4.0 μg/mL plasma paraquat concentration.

| SNP       | Survival N(%) | Non-survival N(%) | OR     | 95% CI       | P-value |
|-----------|---------------|-------------------|--------|--------------|---------|
| C1236T    |               |                   |        |              |         |
| CC        | 7 (19.4%)     | 7 (22.6%)         | 0.828  | 0.255–2.691 | 0.753   |
| TT        | 29 (80.6%)    | 24 (77.4%)        |        |              |         |
| C3435T    |               |                   |        |              |         |
| CC        | 14 (38.9%)    | 10 (32.3%)        | 0.98   | 0.278–3.460 | 0.975   |
| CT        | 10 (27.8%)    | 7 (22.6%)         | 1.633  | 0.533–5.003 | 0.39    |
| TT        | 12 (33.3%)    | 14 (45.2%)        | 1.336  | 0.488–3.662 | 0.573   |
| G2677T/A  |               |                   |        |              |         |
| GG        | 15 (41.7%)    | 13 (41.9%)        | 0.705  | 0.245–2.026 | 0.517   |
| GT or A   | 18 (50.0%)    | 11 (35.5%)        | 0.757  | 0.245–2.026 | 0.517   |
| TT or A   | 3 (8.3%)      | 7 (22.6%)         | 2.692  | 0.575–12.596| 0.208   |
| GT or A+TT or A | 21 (58.3%) | 18 (58.1%) | 0.989 | 0.374–2.618 | 0.982   |
that patients with toxicity. We reanalyzed these patients with a PQ concentration (CC), 37.9–44.6% (CT) of heterozygous, and 43.5–52.1% of homozygous mutants (TT)\(^30,31\). The CT type was mutants compared to that of a previous study. The C1236T frequencies in Asians are 8.3–13.8% of wild type not show pharmacokinetics changes according to MDR1 gene SNPs. Third, our study population included more sion might have affected the function of lung P-gp regardless of the MDR1 polymorphism. Second, we could cant difference in the mortality of these patients. In addition, two or three SNPs in different combinations did not exhibit signifi- cell type \(^132\), it is necessary to reveal the relationship between SNPs and P-gp function in the lung in the future. might be different compared to that in rodents. Therefore, these gene polymorphisms of MDR1 might be able to not included in our study, which might have produced selection bias. Fourth, P-gp expression in human organ intoxicated patients.

In conclusion, our observations suggest that the MDR1 SNPs do not have any effect on the mortality of PQ intoxicated patients.

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### Table 5. Combined distribution of the MDR-1 gene polymorphisms in paraquat intoxicated patients.

|               | Survival N(%) | non-survival N(%) | OR  | 95% CI     | P-value |
|---------------|---------------|-------------------|-----|------------|---------|
| C3435T and C1236T |               |                   |     |            |         |
| C3435T(0) + C1236T(0) | 6   | 11               |     |            |         |
| C3435T(1) + C1236T(0) | 1   | 3                | 1.636 | 0.138–19.387 | 0.696  |
| C3435T(0) + C1235T(1) | 30  | 58               | 1.055 | 0.355–3.130 | 0.924  |
| C3435T and G2677TA |               |                   |     |            |         |
| C3435T(0) + G2677TA(0) | 24  | 32               |     |            |         |
| C3435T(1) + G2677TA(0) | 10  | 29               | 2.175 | 0.891–5.310 | 0.088  |
| C3435T(0) + G2677TA(1) | 3   | 11               | 2.750 | 0.690–10.952 | 0.151  |
| C1236T and G2677TA |               |                   |     |            |         |
| C1236T(0) + G2677TA(0) | 7   | 12               |     |            |         |
| C1236T(1) + G2677TA(0) | 27  | 49               | 1.059 | 0.373–3.007 | 0.915  |
| C1236T(0) + G2677TA(1) | 3   | 11               | 2.139 | 0.440–10.391 | 0.346  |
| C3677T, C1236T and G2677TA |   |                   |     |            |         |
| C3677T(0) + C1236T(0) + G2677TA(0) | 6   | 9                |     |            |         |
| C3677T(1) + C1236T(0) + G2677TA(0) | 1   | 3                | 2.000 | 0.166–24.069 | 0.585  |
| C3677T(0) + C1236T(0) + G2677TA(1) | 0   | 2                |     |            |         |
| C3677T(0) + C1236T(1) + G2677TA(0) | 18  | 23               | 0.852 | 0.256–2.837 | 0.794  |
| C3677T(1) + C1236T(0) + G2677TA(0) | 1   | 1                | 0.667 | 0.035–12.840 | 0.788  |
| C3677T(1) + C1236T(1) + G2677TA(0) | 9   | 26               | 1.926 | 0.535–6.936 | 0.316  |
| C3677T(1) + C1236T(1) + G2677TA(1) | 2   | 8                | 2.667 | 0.414–17.169 | 0.302  |
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Author Contributions
H.J.K., H.-K.K., and J.-T.K. coordinated the experimental work; H.-W.G. coordinated data-analysis and contributed to the writing of the manuscript; S.-H.L., S.E.P., H.-Y.S. and S.-Y.H. contributed to the design of the research plan and organization of the study.

Additional Information

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