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Febuxostat is a non-purine, selective inhibitor of both isoforms of xanthine oxidoreductase (XOR). Its pharmacokinetics is not dependent on renal clearance, and it may be advantageous in patients with chronic kidney disease (CKD). Although febuxostat is effective in patients with mild-to-moderate CKD, its efficacy and safety in patients with severe CKD remain unclear. This retrospective study included patients with an estimated glomerular filtration rate (eGFR) of < 30 ml/min/1.73 m² and hyperuricemia, who received febuxostat. The study was performed at Kaohsiung Medical University Hospital between January, 2015 and December, 2015. Changes were observed in the serum uric acid level, rate of achieving the target uric acid level (<6.0 mg/dL), changes in the eGFR, treatment dosage, and adverse events. 217 patients (65.9 ± 15.1 yrs, 145 males and 72 females) with severe CKD and hyperuricemia were included. Febuxostat significantly lowered the serum uric acid level (9.4 ± 1.9 at baseline and 5.6 ± 1.9 ml/min after treatment, P < 0.001). The serum uric acid level was <6 mg/dL in 126 patients (58.1%). There were no significant changes in eGFR (17.6 ± 7.4 at baseline and 17.8 ± 8.9 ml/min after treatment, P = 0.642) or indices of liver dysfunction. Adverse events were found in 5 patients, all adverse events improved after discontinuing febuxostat. This study demonstrated that febuxostat is efficacious and well tolerated in severe CKD patients with hyperuricemia, although renal function monitoring may be needed.

Key words: Febuxostat, hyperuricemia, renal insufficiency, chronic.

INTRODUCTION

Hyperuricemia is common in patients with chronic kidney disease (CKD). Previous studies have shown that hyperuricemia is associated with an increased plasma creatinine level, which is a risk factor for a low estimated glomerular filtration rate (eGFR), and can affect the progress of renal disease (Sircar et al., 2015). The risk of kidney failure is eight times higher in patients with serum uric acid (sUA) levels >8.5 mg/dl than in those with sUA levels of 5.0 to 6.4 mg/dl (Saag et al., 2016). A previous epidemiological study in Japan showed that hyperuricemia is significantly associated with the incidence of end-stage renal disease (ESRD), and sUA

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levels >6 mg/dl are considered to be an independent predictor of ESRD in women (Iseki et al., 2004).

The 2016 European League Against Rheumatism (EULAR) recommended that the target of urate-lowering treatment should be to achieve sUA levels <6 mg/dl in all gout patients or sUA levels <5 mg/dl in severe gout patients (Richette et al., 2016). Allopurinol is a first-line drug in the treatment of hyperuricemia, and it is mainly excreted via urine. Its metabolite, oxypurinol, may accumulate in the body of patients with insufficient renal function (GFR <50 ml/min/1.73 m²) and produce toxicity; therefore, dosage adjustments are necessary during treatment. However, treatment efficacy is associated with dosage, and a decrease in the dosage may cause difficulty in achieving the treatment goal of controlling sUA levels (Hira et al., 2015).

Febuxostat is a selective inhibitor of xanthine oxidase. It was approved by the US FDA in 2009 for treating gout patients with hyperuricemia (Chohan, 2011). Febuxostat is metabolized mainly in the liver. Previous studies have shown that tolerance toward febuxostat is good in mild-to-moderate renal insufficiency patients. To increase the clinical use and safety of febuxostat, several recent studies focused on its efficacy and safety in patients with severe CKD. However, the number of included patients was low (Saag et al., 2016; Hira et al., 2015; Juge et al., 2017; Shibagaki et al., 2014). The present study assessed the efficacy and safety of febuxostat in controlling hyperuricemia in patients with severe CKD.

**METHODOLOGY**

**Patients**

This retrospective study included severe CKD patients treated with febuxostat at Kaohsiung Medical University Hospital in Taiwan between January 1, 2015 and December 31, 2015. Patient data were collected using the electronic medical record system of the hospital. Patient data included demographic data (age and sex), medical history (mainly focused on cardiovascular and liver diseases), history of gout, and medication record on gout treatment (types of drugs and their dosages). Additionally, data on plasma creatinine levels and changes in the sUA level before and after medication were collected. Since aspartate transaminase is an index for liver function, its value was monitored for adverse reactions. Through follow-up in the outpatient clinic, the adverse reactions of febuxostat during treatment were recorded. This study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUHIRB-E (I)-20170001). This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors. The inclusion criteria were age >20 years, severe CKD (GFR <30 ml/min/1.73 m²), and febuxostat use. The exclusion criteria were sUA levels <6 mg/dl before treatment, acute gout attack within 2 weeks, acute kidney failure, abnormal liver function, kidney transplantation, pregnancy or lactation, and anti-cancer or immunosuppressive treatment.

The treatment goal was set as a sUA level <6 mg/dl within 12 weeks. Changes in the sUA level before and after treatment were recorded, and the overall ratio of patients who achieved the goal and the association between different dosages and their efficacy were analyzed. Changes in serum creatinine levels before and after treatment were compared to evaluate the safety of febuxostat and its effect on the kidneys. eGFR is estimated GFR calculated by the abbreviated MDRD (modification of diet in renal disease) equation: 186 x [(Creatinine/88.4) - 1.154 x (age) - 0.203 x (0.742 if female) x (1.210 if black)]. Serum aspartate transaminase levels over twice the normal value were considered to indicate liver damage. Adverse drug reaction symptoms were monitored, and reports on adverse drug reactions were evaluated for causality using the Naranjo score. If the evaluation result is possible, the possibility cannot be ruled out, but the relative probability is low. If the result is probable, the relative probability is high. In addition, the safety end point was the follow-up cardiovascular event after 24 months.

**Statistical analysis**

Quantitative variables are expressed as mean ± SD, and qualitative variables are expressed in percentage. A paired t-test was used to compare changes in sUA, creatinine, and aspartate transaminase levels before and after treatment. The chi-square test was used to analyze the correlation between age/medical history and post-treatment sUA levels that achieved the treatment goal and for any value <5, the Fisher test was used. The independent sample t-test was used to compare differences in continuous variables within groups. Analysis results with a P-value < 0.05 were considered statistically significant.

**RESULTS**

Between January 1, 2015 and December 31, 2015, 235 patients met the severe CKD diagnostic criteria (eGFR <30 ml/min/1.73 m²) and other study criteria, and of these, 8 patients were kidney transplantation and 10 patients had sUA levels <6 mg/dl before treatment and were thus excluded. Consequently, the study finally included 217 patients, and grouped according to renal function (130 patients with CKD stage 4 and 87 patients with CKD stage 5) (Table 1). The mean patient age was 65.9 ± 15.1 years, and 123 patients (56.7%) were aged >65 years. The study included 7 patients who underwent hemodialysis (HD) and 4 patients who underwent peritoneal dialysis (PD). The percentages of patients with histories of cardiovascular-related diseases are listed in Table 1. Among the included patients, 20 patients had a history of liver disease (diagnosed as chronic hepatitis) and 11 patients had a history of hepatitis B, 2 patients had hepatitis C. The mean alanine transaminase level was 21.8 ± 10.4 IU/L, and there was no acute attack of hepatitis. Within 3 months before treatment, 103 patients (47.5%) did not use any medication for UA. The numbers of patients with gout history are shown in Table 2. The starting dosage of febuxostat was 40 mg/day in 191 patients (88.0%), and the mean dosage was 40.2 ± 9.8 mg/day. The mean sUA level was 9.4 ± 1.9 (range, 6.1 to 18.3) mg/dl before treatment.

**Efficacy**

The mean sUA level after treatment was 5.6 ± 1.9 (range, 1.7 to 9.9) mg/dl. In the study, 126 patients (58.1%)
Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total (N = 217)</th>
<th>CKD stage 4 (N =130) (baseline eGFR&lt;30)</th>
<th>CKD stage 5 (N =87) (baseline eGFR&lt;15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male (%)</td>
<td>145 (66.8%)</td>
<td>88 (67.7%)</td>
<td>57 (65.5%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>65.9 ± 15.1</td>
<td>68.1 ± 14.5</td>
<td>62.9 ± 15.3</td>
</tr>
<tr>
<td><strong>Co morbidities</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>168 (77.4%)</td>
<td>100 (76.9%)</td>
<td>68 (78.2%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>102 (47.0%)</td>
<td>61 (46.9%)</td>
<td>41 (47.1%)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>109 (50.2%)</td>
<td>68 (52.3%)</td>
<td>41 (47.1%)</td>
</tr>
<tr>
<td>Liver disease</td>
<td>20 (9.2%)</td>
<td>17 (13.1%)</td>
<td>3 (3.4%)</td>
</tr>
<tr>
<td><strong>Febuxostat dose</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial dose, mg/day</td>
<td>40.2 ± 9.8</td>
<td>40.9 ± 11.4</td>
<td>39.1 ± 6.8</td>
</tr>
<tr>
<td>Final dose, mg/day</td>
<td>37.7 ± 7.9</td>
<td>37.2 ± 9.2</td>
<td>38.4 ± 5.5</td>
</tr>
<tr>
<td><strong>Baseline serum data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR, ml/min/1.73 m²</td>
<td>17.6 ± 7.4</td>
<td>22.8 ± 4.4</td>
<td>9.9 ± 3.2</td>
</tr>
<tr>
<td>sUA, mg/dL</td>
<td>9.4 ± 1.9</td>
<td>9.3 ± 1.9</td>
<td>9.5 ± 1.7</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>21.8 ± 10.4</td>
<td>21.5 ± 10.5</td>
<td>22.7 ± 10.4</td>
</tr>
<tr>
<td><strong>Final serum data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR, ml/min/1.73 m²</td>
<td>17.8 ± 8.9</td>
<td>23.0 ± 7.0</td>
<td>10.0 ± 4.6</td>
</tr>
<tr>
<td>sUA, mg/dL</td>
<td>5.6 ± 1.9</td>
<td>5.5 ± 2.0</td>
<td>5.6 ± 1.8</td>
</tr>
<tr>
<td>ALT, mean ± SD, IU/L</td>
<td>22.5 ± 15.5</td>
<td>23.5 ± 16.4</td>
<td>21.1 ± 11.7</td>
</tr>
<tr>
<td><strong>CKD cause</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertensive nephropathy</td>
<td>65 (30.0%)</td>
<td>40 (30.8)</td>
<td>25 (28.7%)</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>38 (17.5%)</td>
<td>19 (14.6%)</td>
<td>19 (21.8%)</td>
</tr>
<tr>
<td>Gouty nephropathy</td>
<td>14 (6.4%)</td>
<td>8 (6.1%)</td>
<td>6 (6.9%)</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>3 (1.4%)</td>
<td>1 (0.8%)</td>
<td>2 (2.3%)</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>8 (3.7%)</td>
<td>5 (3.8%)</td>
<td>3 (3.4%)</td>
</tr>
<tr>
<td>Unspecified CKD</td>
<td>89 (41.0%)</td>
<td>57 (43.9%)</td>
<td>32 (36.9%)</td>
</tr>
</tbody>
</table>

*: P-value <0.05 were considered statistically significant
CKD, chronic kidney disease; GI, gastrointestinal; sUA, serum uric acid (mg/dL); ALT, alanine aminotransferase; eGFR, estimated Glomerular filtration rate (ml/min/1.73 m²)

reached the treatment goal (sUA level <6 mg/dl), and 87 of these patients (40.1%) had sUA levels <5 mg/dl. The mean difference in the sUA level before and after treatment was 3.8 ± 0.0 mg/dl, which was statistically significant (P < 0.001) (Figure 1). During the course of treatment, the dosages were adjusted in some patients along with differences in their treatment effects, and the final mean dosage used was 37.7 ± 7.9 mg/day. Patients were divided into two groups based on whether they achieved the treatment goal (sUA < 6 mg/dl). Renal function and sUA levels before treatment, and dosages used were compared between the two groups (Table 3). The mean pre-treatment sUA level (9.9 ± 2.0 vs. 9.1 ± 1.7 mg/dl) were higher and the mean dosage used (39.3 ± 10.9 vs. 40.8 ± 8.9 mg) was lower in the group with post-treatment sUA levels >6 mg/dl.

**Safety**

The mean follow-up eGFR after treatment was 17.8 ± 8.9 (range, 2.9 to 49.4) ml/min/1.73 m². There were no significant changes in eGFR (P = 0.642). In the decline in renal function during and before febuxostat treatment, there was a tendency towards improvement in eGFR during and after febuxostat treatment (Figure 2). The mean follow-up ALT value as an index for liver function was 22.5 ± 15.5 (range, 16 to 82) IU/L. Liver function before and after treatment did not show statistically significant differences (P = 0.481). Five patients
Table 2. Gout history and CKD progression and safety end points.

<table>
<thead>
<tr>
<th>Treatment for hyperuricemia</th>
<th>Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gout history</strong></td>
<td></td>
</tr>
<tr>
<td>Gout arthropathy</td>
<td>84 (38.7)</td>
</tr>
<tr>
<td>Switch from allopurinol</td>
<td>60 (27.6)</td>
</tr>
<tr>
<td>Lack of efficacy</td>
<td>37 (21.6)</td>
</tr>
<tr>
<td>Skin toxicity</td>
<td>10 (4.6)</td>
</tr>
<tr>
<td>Renal function decrease</td>
<td>13 (6.0)</td>
</tr>
<tr>
<td>Use colchicine</td>
<td>50 (23.0)</td>
</tr>
<tr>
<td>Prevent gout acute attack</td>
<td>15 (6.9)</td>
</tr>
<tr>
<td>Treatment gout acute attack</td>
<td>35 (16.1)</td>
</tr>
<tr>
<td><strong>CKD progression</strong></td>
<td></td>
</tr>
<tr>
<td>Before febuxostat treatment</td>
<td></td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>7 (3.2)</td>
</tr>
<tr>
<td>Peritoneal dialysis</td>
<td>4 (1.8)</td>
</tr>
<tr>
<td>Follow-up after one year</td>
<td></td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>31 (14.3)</td>
</tr>
<tr>
<td>Peritoneal dialysis</td>
<td>8 (3.7)</td>
</tr>
<tr>
<td>Follow-up after two years</td>
<td></td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>45 (20.7)</td>
</tr>
<tr>
<td>Peritoneal dialysis</td>
<td>9 (4.1)</td>
</tr>
<tr>
<td><strong>Safety end points (after 24 months)</strong></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td></td>
</tr>
<tr>
<td>Unstable angina</td>
<td>9 (4.1)</td>
</tr>
<tr>
<td>Nonfatal heart failure</td>
<td>12 (5.5)</td>
</tr>
<tr>
<td>Nonfatal myocardial infarction</td>
<td>5 (2.3)</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>4 (1.8)</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>10 (4.6)</td>
</tr>
<tr>
<td>Death from any cause</td>
<td>3 (1.4)</td>
</tr>
</tbody>
</table>

CKD, chronic kidney disease;

experienced adverse reactions (Table 4), and their symptoms showed improvement after treatment discontinuation. Further treatment was discontinued. The safety end point was a follow-up cardiovascular event after 24 months (Table 2).

DISCUSSION

Febuxostat was approved by the US FDA in 2009, for use in the treatment of hyperuricemia-induced gout. On August 1, 2016, the National Health Insurance Administration in Taiwan relaxed the restriction on insurance paid for febuxostat use in gout patients with CKD. However, previous information on the efficacy and safety of febuxostat use in CKD patients above stage 3 is limited. Several studies explored the efficacy and safety of using febuxostat for treating patients undergoing advanced CKD treatment (Table 5) (Sircar et al., 2015; Saag et al., 2016; Hira et al., 2015; Juge et al., 2017; Shibagaki et al., 2014; Sakai et al., 2014). However, because of the limited number of patients included in this study and lower dosages used, further research is required for verifying the results.

Efficacy

With regard to the efficacy of drug treatment, 126 patients (58.1%) achieved sUA levels <6 mg/dl and 87 patients (40.1%) achieved sUA levels <5 mg/dl. The result in this study is lower compared with the finding in the retrospective study by Juge et al (65.1% patients achieved sUA levels <6 mg/dl and 58.9% patients achieved sUA levels <5 mg/dl) (Juge et al., 2017). Nevertheless, dosages used in the two studies were slightly different. In the study by Juge et al (2017), 24.6% of patients used 40 mg/day, 68.1% used 80 mg/day, and
Figure 1. Change in serum uric acid slope (mean ± SD) before and after febuxostat treatment (when is follow up)(number in parentheses indicates number of patients).

Table 3. Differences in clinical characteristics and febuxostat treatment regimen between high and low efficacy groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Last sUA &gt; 6 mg/dL (n = 91)</th>
<th>Last sUA &lt; 6 mg/dL (n = 126)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>68 (74.7%)</td>
<td>77 (61.1%)</td>
<td>0.036*</td>
</tr>
<tr>
<td>Age, years, mean ± SD</td>
<td>64.9 ± 16.0</td>
<td>66.9 ± 14.1</td>
<td>0.319</td>
</tr>
<tr>
<td>Baseline eGFR</td>
<td>17.4 ± 7.1</td>
<td>17.8 ± 7.7</td>
<td>0.750</td>
</tr>
<tr>
<td>Baseline sUA</td>
<td>9.9 ± 2.0</td>
<td>9.1 ± 1.7</td>
<td>0.002*</td>
</tr>
<tr>
<td>Co morbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>72 (79.1%)</td>
<td>96 (76.2%)</td>
<td>0.610</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>42 (46.1%)</td>
<td>60 (47.6%)</td>
<td>0.831</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>47 (51.6%)</td>
<td>62 (49.2%)</td>
<td>0.723</td>
</tr>
<tr>
<td>Liver disease</td>
<td>5 (5.5%)</td>
<td>15 (11.9%)</td>
<td>0.107</td>
</tr>
<tr>
<td>GI symptoms</td>
<td>16 (17.6%)</td>
<td>22 (17.5%)</td>
<td>0.981</td>
</tr>
<tr>
<td>Febuxostat dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial dose, mg/day</td>
<td>39.3 ± 10.9</td>
<td>40.8 ± 8.9</td>
<td>0.283</td>
</tr>
<tr>
<td>Final dose, mg/day</td>
<td>38.2 ± 9.3</td>
<td>37.3 ± 6.9</td>
<td>0.391</td>
</tr>
</tbody>
</table>

*: P-value <0.05 were considered statistically significant
sUA, serum uric acid (mg/dL); SD, standard deviation; eGFR, estimated glomerular filtration rate (mL/min/1.73 m²).

7.3% used 120 mg/day, whereas in this study, most patients (88.0%) used 40 mg/day.
In the study by Lim et al, approximately 76% used 40 mg/day and 23% used 80 mg/day (Lim et al., 2016). The difference in therapeutic dosage may be related to population differences, efficacy results, disease severity of the included patients, and individualities. Additionally, this study excluded patients who experienced acute gout.
Figure 2. Change in estimated glomerular filtration rate slope (mean ± SD) before and after febuxostat treatment (when is follow up) (number in parentheses indicates number of patients).

Table 4. Adverse reactions.

<table>
<thead>
<tr>
<th>Case</th>
<th>Event</th>
<th>Severity</th>
<th>Relation (Naranjo score)</th>
<th>When</th>
<th>Dosage (mg/day)</th>
<th>CKD stage</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Skin rash</td>
<td>Mild</td>
<td>Possible</td>
<td>2 days</td>
<td>40</td>
<td>5</td>
<td>Discontinuation, supported drug</td>
</tr>
<tr>
<td>2</td>
<td>Epigastralgia</td>
<td>Mild</td>
<td>Possible</td>
<td>1 month</td>
<td>40</td>
<td>4</td>
<td>Discontinuation</td>
</tr>
<tr>
<td>3</td>
<td>Vomiting</td>
<td>Mild</td>
<td>Possible</td>
<td>1 month</td>
<td>20</td>
<td>4</td>
<td>Discontinuation</td>
</tr>
<tr>
<td>4</td>
<td>Hepatotoxicity</td>
<td>Mild</td>
<td>possible</td>
<td>1 month</td>
<td>40</td>
<td>4</td>
<td>Discontinuation, ALT &gt; 3 × Normal</td>
</tr>
<tr>
<td>5</td>
<td>Myopathy</td>
<td>Mild</td>
<td>possible</td>
<td>2 years</td>
<td>40</td>
<td>4</td>
<td>Discontinuation</td>
</tr>
</tbody>
</table>

CKD, chronic kidney disease; ALT, alanine aminotransferase

attack within the past 2 weeks and evaluation information on chronic kidney stone was lacking, which may have resulted in the underestimation of disease severity.

Nearly half of the patients (103 patients, 47.5%) did not use any gout medication before the study. Allopurinol requires dosage adjustments; however, its efficacy is correlated with dosage. The adjusted dosage may not be able to achieve the treatment goal. Previous studies have also shown that patients with the HLA-B*5801 gene are more susceptible to allergic reactions; therefore, gene screening is needed before treatment. Consequently, the National Health Insurance Administration in Taiwan relaxed the restriction for febuxostat payment to increase its clinical efficacy and decrease the incidence of adverse reactions.

Patients were divided into two groups based on whether they achieved the treatment goal (sUA <6 mg/dl). The study showed that differences for successful treatment are significantly related to pre-treatment sUA level. However, there was no significant difference between the two groups with regard to age, renal function, and therapeutic dosage. Thus, the outcome difference may be related to patient individuality, disease severity, and combined drug use during treatment. The study by Juge et al. showed that differences for successful treatment is significantly related to gout disease and dosages used (Juge et al., 2017).

Topiroxostat is another novel xanthine oxidoreductase
Table 5. Compared with other studies of febuxostat use in patients with chronic kidney disease.

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Place</td>
<td>India (asian)</td>
<td>Not Asian</td>
<td>Japan</td>
<td>Japan</td>
<td>France</td>
<td>Japan</td>
<td>Taiwan</td>
</tr>
<tr>
<td>Study design</td>
<td>Single-center, double-blind, randomized, placebo-controlled</td>
<td>Multicenter, randomized, double-blind, placebo-controlled</td>
<td>Prospective, open-label, noncontrolled study</td>
<td>Retrospective therapeutic study</td>
<td>Multicenter, retrospective study</td>
<td>Retrospective observational study</td>
<td>Single-center, retrospective study</td>
</tr>
<tr>
<td>Patients</td>
<td>eGFR:15~&lt;60 stage 3 (&lt;60~30): 43</td>
<td>eGFR:15<del>50 stage 4 (&lt;60</del>30): 60</td>
<td>eGFR&lt;45 stage 3b (&lt;45):19</td>
<td>eGFR&lt;60 stage 4 (&lt;60~30): 20</td>
<td>eGFR&lt;30 stage 4 (&lt;30): 60</td>
<td>eGFR&lt;60 stage 4 (&lt;30): 130</td>
<td>eGFR&lt;30 stage 5 (&lt;15): 87</td>
</tr>
</tbody>
</table>

eGFR, estimated glomerular filtration rate (mL/min/1.73 m²); sUA, serum uric acid (mg/dL).

inhibitor; Terawaki et al. (2017) clinically demonstrated that it may be superior to febuxostat since a significant decrease in the urinary protein level was observed. However, as this is an observational study, and is not equivalent to a clinical setting, further research is required for confirming this result (Terawaki et al., 2017).

**Safety**

Renal function was monitored before and after drug treatment, and no significant changes in eGFR were noted ($P = 0.642$). In the present study, eGFR slopes were evaluated for determining the effect of active reduction of serum uric acid concentrations on renal function. Whereas the eGFR slope was negative before febuxostat administration, it became positive after febuxostat treatment (Figure 2). The prospective study by Sircar et al. showed that decreasing sUA levels may slow the progression of CKD (Sircar et al., 2015). Shibagaki et al. analyzed the 6-month treatment effect in patients with CKD at different stages. A finding of the study was that reduction sUA levels were associated with the progression of CKD; the treatment increased the eGFR in stage 3b patients and decreased the eGFR in stage 4 and 5 patients. The overall effect was decreased eGFR, but it did not reach statistical significance (Shibagaki et al., 2014). The present study included 11 dialysis patients (before febuxostat treatment), and follow-up renal function progression after one year and two years (Table 2). The number of patient has increased, but no objective data are present to support whether the outcome is associated with the drug’s effect. Using ≥10% decrease in renal function at the last follow-up as an indication for renal damage, 81 patients (37.3%) showed differences in renal function from the baseline. The report by Juge et al. stated that the renal function of 40% of stage 4 CKD patients and 53.8% of stage 5 CKD patients worsened (Juge et al., 2017). Whether febuxostat has a damaging or protecting effect on the kidneys remains debatable. No objective data is present that supports whether the outcome is associated with the drug’s effect or changes in kidney function. Thus, renal function should be regularly monitored during drug treatment for ensuring safety. No serious adverse reaction occurred during the study, which is similar to the finding in previous study using febuxostat in severe CKD patients (Sircar et al., 2015; Saag et
The results indicate the safety of febuxostat use in CKD patients. Ten patients previously had allergic reactions to allopurinol, and no adverse reaction occurred after switching to febuxostat. The most common adverse reactions to febuxostat were skin rashes, joint pain, nausea, and liver malfunction. Using the Naranjo score for adverse reactions in this study, the evaluation presented all the results as possible, that is, the probability cannot be excluded, but relative probability is low. The study included severe CKD and dialysis patients, and common symptoms included fatigue, weakness, and reduced urine volume. The patient's self-described senses of fatigue and intolerance were subjective feelings, lacking objective data supporting their correlations with the treatment. With regard to liver function, no significant changes in liver function before and after treatment were observed in this study.

In drug safety communication released on November 15, 2017 by the Food and Drug Administration (USA), the incidence of heart-related events caused by febuxostat administration was reported to be higher than that caused by allopurinol administration. In this study, the safety end points were the follow-up of cardiovascular and death events at 24 months (Table 2). The cardiovascular events are nonfatal in nature. Three patients died, but no objective data are present that support the association of this outcome with febuxostat administration. The findings of the clinical study by WB et al. indicate a higher risk of total and cardiovascular mortality with febuxostat than with allopurinol administration. The preclinical studies of febuxostat have shown no cardiovascular toxic effects. A large number of patients discontinued participation in the trial, and in the intention-to-treat analysis includes merely 10% of patients (White et al., 2018). It is therefore recommended that, within the clinical setting, related risk factors should be monitored for increasing the safety of the drug treatment.

The present study had several limitations, mainly because of the lack of comparative data with a control group in its retrospective design. Although whole blood cell counts and biochemical results were regularly monitored, mild adverse reactions may have been overlooked, or the incidence may have been underestimated owing to differences in patient tolerance. In addition, patients who had undergone kidney transplantation were not included. The number of patients was small, and their follow-up time was short. In the future, long-term, large-scale, multi-center controlled studies are needed to explore the efficacy and safety of febuxostat in patients with severe CKD and those undergoing dialysis.

**Conclusion**

The study showed that febuxostat treatment in patients with hyperuricemia and severe chronic kidney disease effectively reduced sUA levels with no significant adverse reaction, and patient tolerance to the drug was good. Whether febuxostat has a damaging or protecting effect on the kidneys remains debatable. Continuous monitoring of related variables is required for increasing the safety of the drug treatment.

**CONFLICT OF INTERESTS**

The author has not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


Full Length Research Paper

Gamma oryzanol loaded microspheres with improved bioavailability

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Gamma oryzanol is a phytosterol that is extracted from rice bran oil. It is an antioxidant that possesses a curative effect for many diseases. Gamma oryzanol is a water insoluble compound supporting the idea that it has a low bioavailability due to low intestinal absorption. In this study, γ-orizanol was loaded in biodegradable microspheres. Intestinal absorption of γ-orizanol from microspheres was compared with that absorbed from triolein solution in rabbits. After oral administration of 150 mg/kg of body weight, plasma was collected at intervals and analyzed for γ-orizanol content using high performance liquid chromatography (HPLC). Results showed that the highest plasma concentration of γ-orizanol, detected from triolein solution, was 6.37 ± 1.48 µg/ml, whereas the highest plasma concentration from γ-orizanol microspheres was 130.30 ± 30.40 µg/ml. This concentration was significantly higher than the amount absorbed from triolein solution (p<0.01). In conclusion, microspheres offer an alternative dosage form for γ-orizanol solution with improved bioavailability.

Key words: Gamma oryzanol, solution, microspheres, bioavailability.

INTRODUCTION

Gamma oryzanol is a natural antioxidant that is extracted from rice bran oil. Crude rice bran oil contains 1599 to 1666 mg γ-orizanol per 100 g (Pattong and Parichat, 2014). It has been found that γ-orizanol has a curative effect for many human diseases, such as improving the symptoms of dementia (Masahiko et al., 2018), enhancing glucose uptake by insulin-resistant cells (Chang et al., 2015), inhibition of platelet aggregation (Cicero and Gaddi, 2001), and reduction of plasma cholesterol level (Wilson et al., 2007). In a study on rats with colon cancer, it was found that γ-orizanol has the capability to improve immunity by increasing the activity of both natural killer and macrophages cells (Kim et al., 2012). It was also found that γ-orizanol is able to reduce melanin concentration by decreasing its synthesis in melanoma cells (Jun et al., 2012). In addition, γ-orizanol
promotes skin capillary, so it has been used in cosmetics industry (Aladedunye and Przybylski, 2013). It is known that plant sterols have limited bioavailability due to their poor water solubility. As such, the effect of γ-oryzanol could be limited by its low bioavailability. Oral route of medicine administration is the most preferable one. However, oral administration limits the bioavailability of medicines to certain degrees according to their physicochemical properties. Gamma oryzanol occurs in a powdered form with low water solubility (Nauman et al., 2017). It is a mixture of ferulic acid esters of triterpene alcohols and plant sterols that are called phytosterols (Patel and Naik 2004). Gamma oryzanol chemical structure indicates that it has a bioavailability problem. Emulsified γ-oryzanol rich fraction proved to enhance its effect in decreasing plasma low density lipoprotein (LDL) and increasing high density lipoprotein (HDL) levels (Aminu et al., 2016). Bulksiness of emulsion dosage forms could be inconvenient for some patients. However, solid carriers may be useful to improve the therapeutic efficacy of lipophilic compounds. Microspheres are small solid envelopes that can carry lipophilic compounds (Zhang et al., 2014). They are spherical particles with diameters ranging from 1 to 100 μm with an ideal particle size less than 200 μm (Alagusundaram et al., 2009). Even small particle size of microspheres can provide a large surface area that can enhance the bioavailability of poorly soluble drugs making them good carriers for poorly such drugs. Modified natural compounds like starches, gums, fats, waxes and protein, natural polymers such as albumin and gelatin, and biodegradable synthetic polymers including poly lactic acid and polyglycolic acid are used as carriers; nevertheless, certain polymers are preferable due to their biocompatibility and biodegradability.

MATERIALS AND METHODS

Chemicals and instruments
Gamma oryzanol was obtained from Tokyo Chemical Industry (TCI) (Tokyo); triolein and poly (D,L-lactide-co-glycolide) (PLGA) from Sigma-Aldrich (St. Louis, MO, USA); chloroform and methanol from BDH (BH15 1TD2, England); sodium lauryl sulfate (SLS) from USA (Fluka chemical, USA); other reagents and solvents either HPLC or analytical grade were purchased from Merck (Darmstadt, Germany). High Performance Liquid Chromatography (HPLC) (HP 1100, Palo Alto, CA); Ultracentrifuge, Beckman L7-65 was purchased from Beckman Instruments (Palo, Alto, CA); Electronic balance, Fy-350, (A&D Company, Ltd, Japan).

Animals
Animal handling was in accordance with the ethical guidelines of the University’s Institutional Animal Care Committee (Approval No. UPM/FPSK/PADS/BR-UUH/00477). Female New Zealand white rabbits, weighing around 1 to 1.5 kg were purchased from Molekular Saintifik Enterprise (Malaysia). They were individually housed in stainless steel cages. Rabbits were acclimatized for two weeks, receiving 100 g a day of standard commercial feed purchased from Federal Flour Mills (FFM) Berhad (Malaysia).

Preparation of γ-oryzanol solution
Gamma oryzanol is a water insoluble compound. Henceforth, triolein, a triacylglycerol carrying three oleic acid molecules, was used as a solvent. Gamma oryzanol solution was prepared by weighing 100 mg of γ-oryzanol on electronic balance and dissolved in 2 g of triolein according to Fujiwara et al. (1983).

Preparation of γ-oryzanol-loaded microspheres
An oil-in-water solvent evaporation method was used to prepare γ-oryzanol loaded microspheres. This method has been revised from previous reports (Yen et al., 2001; Mirakabad et al., 2014). In order to obtain dispersed phase, 100 mg of γ-oryzanol and 100 mg of poly (D,L-lactide-co-glycolide) (PLGA) is dissolved in glycolide/lactide with a ratio of 50/50 in 5 ml of chloroform. The aqueous phase was prepared by dissolving 201.8 mg of sodium lauryl sulfate (SLS) in 100 ml of water. The dispersed phase was slowly mixed with the aqueous phase while being shaken at 150 rpm. After the addition of the dispersed phase, the speed was increased to 250 rpm and the shaking continued at room temperature for 3.5 h until significant amount of chloroform was evaporated. To obtain solidified microspheres, filtration through filter paper (Whatman 2) was carried out followed by washing with 2 ml of cold water twice. Collected microspheres powder was dried in the oven at 50°C for 12 h to evaporate the chloroform completely. To calculate loading percentage of γ-oryzanol in microspheres, three batches were used. Triplicates of 5 mg of microspheres powder from each batch was weighed on F-350 electronic balance and dissolved in 1 ml of chloroform. Samples were vortexed for 30 seconds and filtered using 0.22 μm syringe filters. An aliquot of 20 μl from each sample was injected into HPLC. Same conditions used for γ-oryzanol analysis were used to analyze γ-oryzanol loading in microspheres. Loading percentage was calculated according to the following equation:

\[
\text{Loading Percentage} = \frac{\text{weight of } \gamma\text{-oryzanol}}{\text{weight of microspheres}} \times 100
\]

Administration of γ-oryzanol solution and microspheres
Rabbits were acclimatized for two weeks, receiving 100 g a day of standard commercial feed with free access to water. Before starting the study, rabbits were fasted overnight but were given the free water access. The animals were divided into two groups with three animals per group. One group received γ-oryzanol in triolein solution and the other received γ-oryzanol microspheres. Doses equivalent to 150 mg/kg of γ-oryzanol in the forms of triolein solution and microspheres were given via feeding tube connected with a syringe.

Blood Sampling
Before taking blood samples, ears of rabbits were shaved gently, wiped with 70% ethanol and around 1 ml of blood samples were withdrawn from ear veins into K3 EDTA tubes at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 h. Blood samples were immediately centrifuged at 3000 g for 10 min and plasma from each sample was collected into Eppendorf tubes wrapped with aluminum foil. Plasma samples were kept at -30°C until they were analyzed for γ-oryzanol content.
Figure 1. HPLC chromatogram of γ-orzanol. Components were detected at 325 nm with PDA detector. Separation was carried out at 250 × 4 mm column packed with 5 μm ODS (C18) Hypersil silica. Mobile phase was acetonitrile /methanol/isopropanol (50:45:5) with a flow rate = 1 ml/min. The first peak is cycloartenyl ferulate, the second peak is 24-methylene cycloartanyl ferulate, the third peak is campesteryl ferulate and the fourth peak is sitosteryl ferulate and campesteryl ferulate.

Extraction of γ-orzanol from plasma

The modified method of Folch et al. (1957) was used to extract γ-orzanol from plasma. Plasma was extracted twice since one extraction step could be insufficient to extract plasma γ-orzanol. In this method, 200 µl plasma was diluted with 600 µl distilled water. Plasma samples were then deprotenized by adding 800 µl absolute ethanol and vortexed for 15 s. Aliquots of 800 µl hexane were added to each sample and vortexed for 90 s. Mixtures were then centrifuged at 1000 g for 3 min and hexane layers were collected. Residues were re-extracted with another 800 µl hexane and hexane layers were combined and analyzed for γ-orzanol content using HPLC (Jasco-Borwin, Tokyo) connected with PU-1580 pump (Jasco), using C18-5 µm, 0.25 x 4 mm column (Hewlett Packard, USA). Peaks were detected by UV detector at 325 nm (UV-1575, Jasco). The mobile phase was a mixture of acetonitrile/methanol/isopropanol (50:45:5) with a flow rate of 1 ml/min for 20 min.

Preparation of γ-orzanol standards

An amount of 50 mg γ-orzanol was weighed on an electronic balance, transferred to a 50 ml volumetric flask and dissolved in 5 ml chloroform. The volume was made up to 50 ml with γ-orzanol mobile phases. This concentration (1 mg/ml) was used as the stock solution. Working solutions at concentrations of 0.5, 0.25 and 0.125 mg/ml were prepared by diluting the stock solution with the mobile phase. These concentrations were used to draw the standard curve used to quantify plasma concentrations of γ-orzanol.

Statistical analysis

The data were analyzed using SPSS window program version 11.0. One way ANOVA was used to compare means of γ-orzanol absorbed from solution and microspheres. Results are given as mean ± SD. P-value < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Gamma orzanol absorption from triolein solution

Gamma orzanol in triolein was given as a single oral dose (150 mg/kg body weight) to the rabbits to measure the amount that can be absorbed and circulated. Plasma level of γ-orzanol was quantified using HPLC. Figure 1 shows that four peaks were dominant at retention times of 16.720, 18.294, 19.568 and 22.390 min, respectively. Plasma concentrations for γ-orzanol were calculated using the standard curve equation. As shown in Figure 2, a good linearity was obtained upon plotting the graph of peaks’ area under the curve versus standards concentrations. Figure 3 shows γ-orzanol level in the plasma during 72 h. Results show that 0.48 ± 0.10 µg γ-orzanol/ml plasma was detected after 0.5 h of administration. However, plasma level increased gradually to reach 6.37 ± 1.48 µg/ml after 4 h (Figure 3), and this concentration was the maximum plasma level of γ-orzanol that was detected from γ-orzanol triolein solution. This result supports Fujiwara et al. (1983) who found that maximum level of γ-orzanol and its metabolites reached after 4 h of oral ingestion. Plasma level of γ-orzanol was decreased after that to reach 0.09 ± 0.01 µg/ml after 48 h, and no amount was detected after 72 h as shown in Figure 3, indicating that it was completely cleared from the blood after that time. Fujiwara et al. (1983) had found that γ-orzanol and its metabolites were cleared from rats after 48 h. However, Fujiwara et al. (1983) had used rats not rabbits, and
different animals’ species could result in different metabolism rate. No recent studies are available about amount of γ-oryzanol absorbed in human or animals. However, Lubinus et al. (2013) had found in a clinical study that 80% of orally administered γ-oryzanol was excreted in human feces.
Gamma oryzanol absorption from microspheres

Among biodegradable polymers, poly(lactic-co-glycolic acid) (PLGA) copolymers have suitable biodegradability and biocompatibility properties (Sonam et al., 2013), which make them the best choice for this study. They are polyesters with properties, which depend on polymer composition, molecular weight, hydrophobicity, crystallinity, surface charge and the nature of coating material (Sonam et al., 2013). For this work, copolymer with 50:50 molar composition of lactic acid and glycolic acid was used since the mixture degrades by 1 week, while other ratios had been found to have longer degradation time (Jain, 2000). The method that was used to load γ-oryzanol in microspheres was oil in water emulsion/solvent evaporation process, which is the primary method that had been used to encapsulate lipophilic drugs into PLGA microspheres (Mirakabad et al., 2014). Loading percentage of γ-oryzanol in PLGA microspheres was calculated using HPLC. Results showed that γ-oryzanol was well loaded into PLGA with a percentage of 63.3 ± 5.9%. Solubility of drugs both in polymer and water as well as their molecular weight are the main factors that affect loading in PLGA (Perugini et al., 2003). Gamma oryzanol is a water insoluble compound and this could be a reason for its high loading percentage.

Amount containing 150 mg γ-oryzanol/kg body weight was given orally to rabbits, and plasma γ-oryzanol level was quantified using HPLC. Results in Figure 4 shows that after 0.5 h, plasma concentration of γ-oryzanol was 19.33 ± 5.15 µg/ml. This concentration was significantly higher \((p < 0.01)\) than plasma γ-oryzanol concentration from triolein solution at the same time, which was 0.48 ± 0.10 µg/ml (Figure 3). Figure 4 shows that plasma level of γ-oryzanol increased gradually until 4 h after ingestion, when the maximum concentration of γ-oryzanol reached 130.30 ± 30.40 µg/ml. Plasma level of γ-oryzanol decreased gradually to reach 6.20 ± 0.60 µg/ml at 72 h (Figure 4), indicating that microspheres also sustained the level of γ-oryzanol in plasma. PLGA polymers have been used as a good delivery system for many drugs to sustain and control their release (Yihan et al., 2016). The maximum concentration of γ-oryzanol from triolein was only 6.37 ± 1.48 µg/ml as shown in Figure 3, which was significantly lower \((p < 0.01)\) than the level from γ-oryzanol loaded microspheres.

Conclusion

In this study, it was found that upon loading γ-oryzanol in microspheres, its bioavailability was significantly improved in comparison with its triolein solution. In addition, polymeric materials used in microspheres preparation have the capability to keep plasma concentration for γ-oryzanol for longer time, since it is released slowly by the decomposition of the polymers. Improvement of γ-oryzanol bioavailability leads to improving its therapeutic efficacy. Loading γ-oryzanol in microspheres will also contribute in improved patient’s compliance since it possess a sustained release pattern.
as such, low doses frequency is needed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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