Effect of oral CS on pruritus and serum tryptase level


Received for publication: 6.4.09; Accepted in revised form: 25.11.09

doi: 10.1093/ndt/gfp628
Advance Access publication 10 December 2009

**Effect of oral cromolyn sodium on CKD-associated pruritus and serum tryptase level: a double-blind placebo-controlled study**

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**Abstract**

**Background.** Generalized pruritus is a significant complication in end-stage renal disease patients. The mechanism is unknown and most treatments are ineffective. This study is the first clinical trial designed to evaluate the effect of cromolyn sodium (CS) on renal itch.

**Methods.** Sixty-two haemodialysis (HD) patients with pruritus were enrolled into the study and were randomly assigned to receive CS or placebo (135mg three times daily) for 8 weeks. Patients were asked to record the severity of their pruritus on each dialysis session on a visual analogue scale (VAS) during the 8 weeks of treatment and 4 weeks following discontinuation of treatment. Serum tryptase levels were determined at baseline, after 8 weeks of treatment and 4 weeks after discontinuation of treatment.

**Results.** Data were analysed in 21 patients in the CS group and 19 patients in the placebo group that completed the study. A significant difference was seen in the severity of pruritus between the two groups during the period of study. Level of pruritus decreased from 8.48±2.2 to 0.9±1.8 after 8 weeks of treatment with CS. Geometric mean of serum tryptase at baseline and 8 weeks after treatment were 21.3 and 19.5 ng/ml for the CS group and 18.03 and 18.2 ng/ml for the placebo group, respectively. Although the geometric mean of tryptase had decreased in the CS group, this decrease was not statistically significant (P=0.214).

**Conclusion.** CS can significantly reduce the severity of pruritus in HD patients, but this effect is not due to a decrease in serum tryptase level.

**Keywords:** cromolyn sodium; haemodialysis; pruritus; tryptase
Introduction

Pruritus is considered a major problem in patients with end-stage renal disease (ESRD) [1]. The prevalence of chronic kidney disease (CKD)-associated pruritus was 42% in the largest and most recent epidemiologic study to date [2]. Similarly, in a study performed on 167 haemodialysis (HD) patients in Iran, 41.9% of patients suffered from pruritus in whom 37% were considered as severe [3]. The pathophysiologic mechanism remains poorly understood. Current theories include: a derangement in the immune system [4], imbalance in the endogenous opioidergic system [5], increased serum calcium and phosphate levels [6,7], abnormal cutaneous innervations, xerosis, abnormalities in affective pain fibres [8], hypervitaminosis A [9], alterations in the number of skin mast cells [10,11], allergic sensitization [12], hyperparathyroidism [11,13] and high levels of BUN [14], histamine [15,16] and substance P [17].

Interactions between dermal mast cells and afferent C neuron terminals may play an important role in the mediation of pruritus since these structures sit very close together. The skin of CKD patients with pruritus has a greater number of mast cells [10,18]. There are various substances released from mast cells including histamine, IL2, TNF-α and proteases such as tryptase [19,20]. Although Stockenhuber et al. found that patients with pruritus undergoing HD had an increased plasma histamine level compared to those without pruritus [16], Mettang et al. demonstrated no relationship between plasma histamine and pruritus score in patients undergoing dialysis [21]. In addition, antihistamines are relatively ineffective in treating uraemic itch. Therefore, it was hypothesized that probably other mediators released from mast cells are responsible for CKD-associated pruritus; tryptase being one of these mediators [19]. Dugas-Breit et al. demonstrated a significant correlation between the intensity of pruritus and serum tryptase levels in HD patients [19]. Mast cell tryptase activates protease-activated receptors (PAR) on sensory neurons, causing neuronal excitation and release of substance P [22,23]. Steinhoff et al. have shown in their experiment that neuronal PAR-2 is involved in pruritus of human skin and that a histamine-independent, protease-dependent, PAR-2-mediated itch pathway may provide a new link to novel therapies for pruritus and cutaneous inflammation [24].

Based on the facts that cromolyn sodium (CS) is a mast cell stabilizer, its oral administration has been effective in the treatment of systemic mastocytosis [25] and urticaria pigmentosa [26,27], and that there have been case reports of CS leading to a reduction in pruritus in two HD patients suffering from severe refractory pruritus [28], we decided to design a placebo-controlled clinical trial to evaluate the antipruritic effect of CS on HD patients. We hypothesized that CS may decrease the severity of pruritus via decreasing serum tryptase levels in HD patients.

Materials and methods

This was a double-blind placebo-controlled trial conducted between August 2008 and June 2009. This study was approved by the ethics committee of Shiraz University of Medical Sciences. All HD patients attending the two major referral hospitals in Shiraz were evaluated. Eligible subjects were adults over 18 years of age who were suffering from pruritus during the past 6 weeks, had not responded to drugs and signed the informed consent. Patients with dermatologic, liver or metabolic diseases associated with pruritus were excluded. Any medication that had antipruritic effect was discontinued 1 week before the study. Patients were randomly assigned into two groups using the stratified randomization method where the prognostic factor was the gender variable. CS was formulated into capsules (size 2) using 135 mg of cromolyn powder (Cambrex Profarmaco, Italy) plus 30 mg lactose powder (Merck-Germany) as filler. Placebo capsules were filled with lactose powder. A hand-operated capsule-filling machine was used.

Drug packages were prepared by the principal investigator (G.V.). Both the participants and the investigator that administered the interventions and assessed the outcomes were blinded to group assignment. Code breaking was performed at the end of data analysis.

The standard dosing of CS for systemic mastocytosis is 200 mg four times daily. However, since the drug has a renal excretion (30–50%), the manufacturer recommends a decrease in dosage in ESRD patients (100 mg four times daily). It is recommended to open each capsule and dissolve its contents in hot water, then add cold water and swallow the liquid solution half an hour before each meal. In this study, we decided to decrease the frequency of drug intake to three times per day in order to increase patient compliance due to the fact that ESRD patients consume a significant number of medications. Therefore, a 135 mg capsule was dissolved in a minimal amount of water (to prevent volume overload) and administered half an hour before each meal. Drug administration was continued for 8 weeks.

HD was performed for 4.5 h two to three times per week via a low-flux polysulphone dialyser [0.8–1.3 m² surface area (Fresenius 4008B, Fresenius Medical Care, Bad Homburg, Germany)] using bicarbonate dialysis fluid. Blood flow and dialysate flow were 250–300 and 500 ml/min, respectively.

Patients were asked to record the severity of their pruritus on each dialysis session (two to three times per week) on a visual analogue scale (VAS). A '0' score represented absence of pruritus and a '10' represented the greatest severity of symptoms. The scores were collected by the same investigator that administered the interventions. The mean pruritus score was recorded for each patient every week for 8 weeks (period of drug administration) and continued for 4 weeks after drug discontinuation.

Pre-dialysis blood samples were drawn for determination of haemoglobin, calcium, phosphorus, albumin, ferritin, parathyroid hormone, white blood cells and serum tryptase at the beginning of the study. A repeated sample was drawn for serum tryptase measurement after 8 weeks of treatment and 4 weeks after drug discontinuation. Tryptase concentration was measured by a fluoroenzyme immunoassay (UniCAP® Tryptase, Pharmacia Diagnostics). The assay detects both α- and β-tryptase. Serum tryptase concentrations above 11.4 μg/L were considered raised. According to the manufacturer, the 95th percentile of serum tryptase concentration in normal subjects is 11.4 μg/L, while the geometric mean is 3.8 μg/L.

Data analysis

All continuous variables are reported as the mean±SD and compared using Student's t-test. Categorical variables are reported as percentages and compared using chi-square test. Multifactorial repeated-measures analysis of variance was used for the overall comparison of the CS and placebo groups during the period of study. The non-parametric Mann–Whitney U-test was used for comparing the severity of pruritus and geometric means between CS and placebo groups. A comparison of the level of pruritus and tryptase levels at different time points within each group was performed using Wilcoxon’s test. Non-parametric Spearman’s correlation was used to determine the association between the severity of pruritus and serum tryptase level. All data were analysed using SPSS version 12.

Results

All dialysis patients (N=205) attending the two major referral hospitals in Shiraz were assessed for eligibility. Six-
ty-two patients fulfilled the inclusion criteria and were randomized in a double-blind fashion into two groups. A flow diagram of the progress through different phases of the trial is shown in Figure 1. Twenty HD patients were included as negative control for the measurement of baseline serum tryptase. Data were analysed in 21 out of 32 patients in the CS group, 19 out of 30 patients in the placebo group, and 19 patients in the negative control group. Reasons for drop out are indicated in the diagram for both groups. All adverse effects were gastrointestinal (GI) and consisted of nausea and diarrhoea. In the CS group, only one patient had adverse effect (flatulence), although this did not lead to drug discontinuation. In the placebo group, six patients experienced adverse effects (two cases had nausea, one case had diarrhoea and three cases experienced both nausea and diarrhoea). Non-compliance was due to the fact that the patients did not get a rapid response and got disappointed in continuing the medication. Patient characteristics in the two groups are listed in Table 1.

The mean pruritus score was 8.48±2.2 (range 4–10, median 10) in the placebo group and decreased to 5.58±3.8 (range 0–10, median 6) after 8 weeks of therapy ($P=0.004$). The mean pruritus score was 8.68±1.8 (range 4–10, median 10) in the CS group which decreased to 0.9±1.8 (range 0–6, median 0) after 8 weeks ($P<0.001$). Repeated-measures analysis of variance showed that there was a significant difference in pruritus scores between the two groups during the period of study ($P<0.001$). Figure 2 compares the severity of pruritus between the two groups during the period of drug administration and 4 weeks after drug discontinuation. The decrease in pruritus score was significant from baseline to Week 1, Week 1 to 2, and Week 2 to 3 (all $P$-values $<0.001$) in the group receiving CS. The decrease was not significant after Week 3, indicating that the drug had its maximum effect after this period of time. Fifteen out of 21 patients (71%) that received CS had a pruritus score of ‘0’ (no symptoms) after 3 weeks, while only five out of 19 (26%) patients receiving placebo were symptom-free after this period.

Baseline serum tryptase levels were evaluated in 36 HD patients with pruritus and 18 patients in the control group. Geometric mean of tryptase in all HD patients (with and
without pruritus, N=54) was 17.65 ng/ml. Serum tryptase concentration was raised above the 95th percentile in 91% of HD patients with pruritus, while only 68% of HD patients without pruritus had tryptase levels above this percentile. The geometric mean of serum tryptase was 19.6 ng/ml in HD patients suffering from pruritus and 14.3 ng/ml in patients without pruritus, while only 68% of HD patients with severe pruritus resistance to other drugs, CS was administered and there was improvement in symptoms within 2 weeks, reaching a maximum after 12 weeks. Within 4 weeks after drug discontinuation, pruritus symptoms significantly worsened [28]. In the present study, improvements were seen after 1 week, reaching its maximum effect after 3 weeks.

Although the decrease in symptoms was seen in both the CS and placebo groups, there was a significant difference between the pruritus scores in the two groups at different time points, leading to the fact that CS is significantly more effective than placebo. Interestingly, this effect was sustained for 4 weeks after drug discontinuation, which proposes a long duration of action for this drug.

Compared to gabapentin that has been proven to be effective for the treatment of CKD-associated pruritus, CS has less adverse effects. In the double-blind placebo-controlled trial on gabapentin, somnolence, dizziness and fatigue were common side effects noticed during the trial [29]. Our patients did not have any side effects with CS except for one case who complained of flatulence. Some patients in the placebo group had GI side effects (nausea and diarrhoea) which were probably due to lactase deficiency.

Soter et al. hypothesized that CS acts by mechanisms other than stabilization of mast cell membranes, since histaminuria and eosinophilia persisted in the face of an effective clinical response [25]. Rukwied et al. hypothesized that mast cell mediators other than histamine induce pruritus in patients with atopic dermatitis (AD). In this study, mast cell-derived tryptase and chymase were considered as possible candidates for mediating mast cell-induced pruritus in AD patients [30]. Dugas-Breit et al. concluded that tryptase may be involved in the pathogenesis of pruritus in HD patients [19].

### Discussion

Therapy with the mast cell stabilizer, ketotifen, has been associated with significant improvement in pruritus in a small study [15]. CS is a well-known mast cell stabilizer that is proven to be effective in systemic mastocytosis [25]. There have also been reports of its effectiveness in urticaria pigmentosa [26,27]. In the study performed by Lidskov et al. on the effects of oral CS on urticaria pigmentosa, the antipruritic effect was observed after 12 h to 2 weeks [26], in contrast to Czannetzki and Behrendt [27] and Soter et al. [25] who first noticed the antipruritic effect after 2 to 6 weeks in patients with urticaria pigmentosa and systemic mastocytosis. In two case reports presented by Rosner on two HD patients with severe pruritus resistance to other drugs, CS was administered and there was improvement in symptoms within 2 weeks, reaching a maximum after 12 weeks. Within 4 weeks after drug discontinuation, pruritus symptoms significantly worsened [28]. In the present study, improvements were seen after 1 week, reaching its maximum effect after 3 weeks.

### Table 1. Patient demographics and laboratory data at the beginning of the study

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Placebo (N=19)</th>
<th>Cromolyn (N=21)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
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<tr>
<td>Weight (kg)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
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</tr>
<tr>
<td>Sex</td>
<td>N (%)</td>
<td>N (%)</td>
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</tr>
<tr>
<td>Female</td>
<td>11 (57)</td>
<td>9 (42)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8 (43)</td>
<td>12 (58)</td>
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<tr>
<td>Duration of dialysis (year)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td>Baseline pruritus (VAS)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td>Frequency of dialysis per week</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td>Kt/V per dialysis (single pool)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td>PTH (pg/ml)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td>WBC (count/dL)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td>RBC (count/dL)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
</tr>
<tr>
<td>Hgb (g/dL)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
</tr>
<tr>
<td>Hct (%)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td>Platelet (count/dL)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td>Cr (mg/dL)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
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<tr>
<td>Alb (g/dL)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td>Serum ferritin (ng/mL)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td>Calcium (mg/dL)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td>Phosphate (mg/dL)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
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</tbody>
</table>

SD, standard deviation; N, number; VAS, visual analogue scale; PTH, parathyroid hormone; WBC, white blood cell; RBC, red blood cell; Hgb, haemoglobin; Cr, creatinine; Htc, haematocrit; Alb, albumin.

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Similar to the study performed by Dugas-Breit et al., tryptase levels in our HD patients were high (17.65 ng/ml) compared to the normal population (3.8 ng/ml) and even higher in HD patients with pruritus compared to those without pruritus (19.6 and 14.3 ng/ml, respectively, \( P=0.036 \)). However, the non-parametric Spearman's correlation did not show a significant association between pruritus intensity and tryptase level (Spearman's coefficient = 0.263, \( P=0.054 \)). In the study performed by Dugas-Breit, Pearson's correlation was performed and showed a significant correlation between pruritus intensity and tryptase level (Pearson's coefficient = 0.3, \( P=0.014 \)). It should be noted that our Spearman's correlation \( P \)-value is very close to being significant.

Although we hypothesized that the antipruritic effect of CS is due to a decrease in serum tryptase levels, our study did not support this hypothesis. Serum tryptase levels were not significantly decreased by CS administration. This leads to the fact that there should be other mechanisms involved in the antipruritic effect of CS.

Probably, other mast cell mediators are involved in CKD-associated pruritus and that CS affects these mediators. Chymase is a chymotrypsin-like serine protease stored within the mast cell granules and secreted upon stimulation of the cells. Mast cell chymase might have a role in CKD-associated pruritus.

Another proposed mechanism could be that, that CS may affect tissue levels of tryptase rather than its serum level. Future studies on the pathogenesis of pruritus in dialysis patients must include determination of tissue levels of tryptase and other mast cell mediators.

It is possible that CS has a local effect on the GI tract. Less than 1% of the dose is absorbed when CS is administered orally. This leads us to the fact that CS may act through stabilization of the GI mucosal mast cells and, therefore, decreases intestinal tryptase levels without affecting serum levels. In a study performed by Moeser et al. on GI dysfunction induced in pigs, mast cell tryp-
tase was enhanced in the colonic mucosa, and remained elevated without affecting the serum levels of tryptase. The authors suggested that, once released from the mast cell, tryptase remains in the intestinal mucosa [31]. It should also be noted that there may be other factors released from the GI tract that can induce pruritus in the CKD population [32] and CS may prevent the release of these factors. Finally, the antipruritic effect of CS may be due to mechanisms other than mast cell stabilization. Although it is believed that CS is a mast cell stabilizer, its mechanism of action is not fully understood. For example, drug companies have produced 20 related compounds with mast cell-stabilizing activity equal to or even more than CS, but none of them had any anti-asthmatic effect (http://en.wikipedia.org/wiki/Cromoglicic_acid). It has been suggested that CS inhibits sensory nerve activation [33]. This may be due to the inhibition of chloride channels [34,35], reduction of neuropeptide release from nerves [36] or tachykinin receptor antagonism [37]. Whether these effects remain at play after oral administration of the drug remains a matter of debate due to its poor intestinal absorption as mentioned above.

We report a double-blind placebo-controlled trial to assess the effectiveness of oral CS in CKD-associated pruritus for the first time. Our study clearly shows the impressive effectiveness of the drug. However, the mechanism of action is not due to a decrease in serum tryptase level and still remains to be understood.

Acknowledgements. This research was supported by Shiraz University of Medical Sciences (grant no. 4146).

Conflict of interest statement. None declared.

References


Table 2. Geometric means of serum tryptase at different time points: baseline, after 8 weeks of treatment and 4 weeks after drug discontinuation

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Geometric mean (ng/ml)</th>
<th>P-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline serum tryptase CS (N=18)</td>
<td>21.3</td>
<td>0.214</td>
</tr>
<tr>
<td>Placebo (N=18)</td>
<td>18.03</td>
<td></td>
</tr>
<tr>
<td>Serum tryptase levels 8 weeks CS</td>
<td>19.5</td>
<td>0.802</td>
</tr>
<tr>
<td>Placebo (N=17)</td>
<td>18.22</td>
<td></td>
</tr>
<tr>
<td>Serum tryptase levels 4 weeks CS</td>
<td>16.84</td>
<td>0.616</td>
</tr>
<tr>
<td>Placebo (N=16)</td>
<td>17.7</td>
<td></td>
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</table>

aMann–Whitney U-test.
Variation in IGHMBP2 is not associated with IgA nephropathy in independent studies of UK Caucasian and Chinese Han patients

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Abstract

Background. IgA nephropathy is a major cause of end-stage renal disease worldwide. Its aetiology is poorly understood but there is good evidence for a major genetic component, although to date, no gene has been conclusively identified. We describe a new UK multicentre DNA collection assembled to investigate this. A Japanese genome-wide analysis recently reported that common genetic variation in immunoglobulin mu-binding protein 2 (IGHMBP2) was associated with IgA nephropathy. We sought to replicate this using the new UK collection, and through an independent parallel analysis of a Han Chinese population.

Methods. In the UK collection, haplotype-tagging (tag) single-nucleotide polymorphisms (SNPs) and haplotypes were analysed in a case-control study (349 cases, 605 controls) and family-based analysis (162 complete and 23 partially complete family trios), which was performed using the transmission disequilibrium test. In parallel, 663 cases of IgA nephropathy and 663 controls from a Chinese population were analysed: coding and flanking regions of the gene were re-sequenced in a subset, and SNP and haplotype association analysis was performed in the whole collection using the identified tagSNPs and all the coding and exonic flanking SNPs.