New treatment for IgA nephropathy: enteric budesonide targeted to the ileocelecal region ameliorates proteinuria

Hilde Kloster Smerud1, Peter Bárény2, Karin Lindström2, Anders Fernström3, Anna Sandell3, Peter Pählsson4 and Bengt Fellström1

1Department of Medical Sciences, Division of Nephrology, Uppsala University Hospital, University of Uppsala, Uppsala, Sweden, 2Department of Renal Medicine, CLINTEC, Karolinska Institutet, Stockholm, Sweden, 3Department of Nephrology, Linköping University Hospital, University of Linköping, Linköping, Sweden and 4Department of Clinical and Experimental Medicine, University of Linköping, Linköping, Sweden

Correspondence and offprint requests to: Hilde Kloster Smerud; E-mail: hilde.smerud@medsci.uu.se

Abstract

Background. Systemic corticosteroid treatment has been shown to exert some protection against renal deterioration in IgA nephropathy (IgAN) but is not commonly recommended for long-term use due to the well-known systemic side effects. In this study, we investigated the efficacy and safety of a new enteric formulation of the locally acting glucocorticoid budesonide (Nefecon®), designed to release the active compound in the ileocecal region. The primary objective was to evaluate the efficacy of targeted release budesonide on albuminuria.

Methods. Budesonide 8 mg/day was given to 16 patients with IgAN for 6 months, followed by a 3-month follow-up period. The efficacy was measured as change in 24-h urine albumin excretion, serum creatinine and estimated glomerular filtration rate (eGFR).

Results. The median relative reduction in urinary albumin excretion was 23% during the treatment period (interquartile range: −0.36 to −0.04, P = 0.04) with pretreatment values ranging from 0.3 to 6 g/24 h (median: 1.5 g/24 h). The median reduction in urine albumin peaked at 40% (interquartile range: −0.58 to −0.15) 2 months after treatment discontinuation. Serum creatinine was reduced by 6% (interquartile range: −0.12 to −0.02; P = 0.003), and eGFR [Modification of Diet in Renal Disease (MDRD)] increased −8% (interquartile range: 0.02–0.16; P = 0.003) during treatment. No major corticosteroid-related side effects were observed.

Conclusions. In the present pilot study, enteric budesonide targeted to the ileocecal region had a significant effect on urine albumin excretion, accompanied by a minor reduction of serum creatinine and a modest increase of eGFR calculated by the MDRD equation, while eGFR calculated from Cockcroft–Gault equation and cystatin C was not changed. Enteric budesonide may represent a new treatment of IgAN warranting further investigation.

Keywords: budesonide; clinical trial; corticosteroid; IgA nephropathy; prospective

Introduction

IgA nephropathy (IgAN) is the most common glomerulonephritis worldwide. Because of the varying clinical presentation, no consensus for immunomodulating treatment of IgAN has been established. Blockade of the renin-angiotensin-aldosterone system (RAAS) with angiotensin-converting enzyme inhibitors (ACEI) and/or angiotensin-II receptor blockers (ARB) is recommended as they have renoprotective and antiproteinuric effects [1]. In a meta-analysis of studies, in primary glomerulonephritis, combination therapy with ACEI and ARB has shown additional renoprotective effects compared to single RAAS blockade [2]. Omega 3 fatty acids may have a modest effect on disease progression, although the results are not conclusive [3]. Systemic corticosteroids have in some studies shown reduction in proteinuria and protection against renal deterioration [4–8] but are not generally recommended for long-term use due to the well-known systemic side effects [9]. Other therapeutic options are still on an experimental level.

IgAN is characterized by mesangial deposits of polymeric IgA and altered mucosal IgA responses may be one underlying mechanism of the disease. For our study, a new enteric capsule formulation of the locally acting glucocorticoid budesonide (Nefecon®) was designed using a modification of the TARGIT starch capsule technology [10] to release the active compound in the distal part of the ileum and the proximal part of the colon where the Peyer’s patches are located. Budesonide is absorbed in the ileum but undergoes first pass metabolism (via CYP3A4 in the liver) to compounds (16α-hydroxybudesonide and 6-beta-hydroxybudesonide) having low glucocorticoid activity. It is hypothesized that such targeted release of budesonide will exert its effects by local immunosuppression and suppression of immune complex formation and that the local administration will minimize the systemic side effects seen with oral corticosteroids. The aim of the present study was...
to explore the effect of targeted budesonide on albuminuria and glomerular filtration rate (GFR).

Materials and methods

Patients
A total of 16 patients (10 males) with biopsy-proven IgAN were recruited from three Swedish University hospitals (Uppsala University Hospital, Karolinska Institutet and Linköping University Hospital). The mean age for the patient group was 39 ± 13 years (range: 21–74). All patients were ≥18 years, had an albuminuria (U-albumin) of >500 mg/day and serum creatinine (S-creatinine) <200 µmol/L (verified by at least four sample results within a 2-year period prior to inclusion). All patients gave their written informed consent to participation prior to study inclusion.

The main exclusion criteria were severe gastrointestinal disorders which could impair drug effect or other conditions which could modify the pharmacological effects of the study drug as determined by the investigator; uncontrolled blood pressure (treated or untreated) defined as a systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥100 mmHg; hyperlipidaemia defined as unacceptable levels of lipids according to the investigator; introduction of an ACE inhibitor, ARB or other blood pressure lowering substance within the 3 months immediately prior to enrolment; treatment with immunosuppressive or systemic corticosteroid agents; intake of CYP3A4 inhibitors (including grape fruit juice); severe liver disease (defined as ASAT and/or ALAT and/or γ-GT values greater than twice the upper limit of normal); uncontrolled (treated or untreated) congestive heart failure as judged by the investigator; current or history of malignancies during the last 3 years; history or presence of psychological or psychiatric illness which could interfere with the patient’s ability to adhere to the protocol; present alcohol or drug abuse; intake of other investigational drug within 30 days prior to enrolment. Women of childbearing potential were required to implement adequate contraceptive measures during the study.

Fourteen patients received stable doses of ACE inhibitors (nine patients) and/or ARBs (eight patients) prior to and during the study. One patient received diuretics in addition to an ACE inhibitor.

Investigational medicinal product
Nefcon® (PharmalINK AB, Stockholm, Sweden) is a targeted release formulation of the corticosteroid budesonide, based on starch capsules, pH-sensitive coatings and sugar beads [TARGET® technology (Archimedes Pharma Ltd, Reading, UK)]. Each capsule contains 4 mg budesonide and is designed to release its content in the ileocecal region of the gastrointestinal tract.

Study design
The study was an open-label, uncontrolled proof-of-concept study, designed to explore the potential treatment effect and safety profile of targeted release budesonide. Budesonide 8 mg/day was given orally for 6 months, followed by a 3-month follow-up period. Doses of ACE inhibitors and ARBs were not changed during the study. The efficacy was measured as change in 24-h urine albumin excretion, serum creatinine and estimated glomerular filtration rate (eGFR) (determined both from the levels of plasma cystatin C and calculated by using the Modification of Diet in Renal Disease (MDRD) formula and the Cockcroft–Gault formula). Serum concentrations of IgA and IgA antibodies against glidin were determined by enzyme-linked immunosorbent assay at baseline and at the end of treatment or the time of early withdrawal from treatment.

Measurement of Gal-deficient IgA1 was performed both before and after removal of sialic acid by neuraminidase treatment essentially as described by Moldoveanu et al. [11]. Ninety-six-well MaxiSorp plates (NUNC A/S, Roskilde Denmark) were coated overnight at 4°C with 10 µg/mL Fab’ fragment of goat IgG anti-human IgA (Jackson ImmunoResearch Labs, West Grove, PA) in coating buffer (15 mM NaCO3, 35 mM NaHCO3, pH 9.6). Non-specific binding was blocked with phosphate-buffered saline-0.05% Tween (PBS-T) containing 2% bovine serum albumin for 4 h followed by incubation with serum samples diluted with PBS-T to a concentration of 100 µg/mL IgA overnight at room temperature. After washing, biotin-labelled GalNAc-binding lectin from Helix aspersa (HAA; Sigma-Aldrich, St Louis, MO) was added to each well (diluted 1:200 in PBS-T). After 3 h of incubation at 37°C, the plates were washed and the wells were incubated with ExtrAvidin (Sigma) diluted 1:10 000 in PBS-T for 1 h at room temperature. After incubation, the plates were washed and substrate solution was added to each well (O-phenylenediamine-H2O2, Sigma). Colour reaction was stopped using 0.5 M sulphuric acid and the absorbance was recorded at 490 nm. All washes were performed with PBS-T.

The same protocol was used for analysis of desialylated samples except that neuraminidase from Clostridium perfringens (2 mU/mL, Sigma) in 5 mM acetate buffer, pH 5.5, was added to each well and incubated at 37°C for 3 h prior to adding biotinylated HAA.

Safety was assessed by measuring haematology parameters, liver enzymes, electrolytes and vital signs and by paying particular attention to potential systemic side effects of corticosteroids. Spontaneously reported adverse events were also recorded.

The primary objective of this study was to evaluate the efficacy of targeted release budesonide on albuminuria in patients with IgAN. The secondary objectives were to investigate the effect of budesonide on GFR and serum creatinine and to assess the product safety.

Statistical analyses
The results are presented with the median and interquartile range within parentheses. The Wilcoxon signed-rank test was used to test if the relative change from baseline to the end of treatment differed from zero. Regression analyses were performed to estimate the individual slope before and during treatment. The slopes during treatment and the difference between slopes before and during treatment were then tested if they differed from zero using the Wilcoxon signed-rank test. Significance of the latter test indicates that there is a change in individual slope during treatment adjusted for individual slope before treatment. For statistical analyses, R version 2.7.2 (R foundation for statistical computing, Vienna, Austria) and SAS version 9.1.3 (SAS Institute Inc., Cary, NC) were used.

Ethics and administration
The study was approved by the Swedish Medical Products Agency and the Ethics Committee of the Medical Faculty, Uppsala University, and performed in accordance with Good Clinical Practice and the Declaration of Helsinki.

Results
The median reduction in urine albumin was 529 mg/24 h (interquartile range: −60 to −757), i.e. a relative reduction of 23% from baseline until the end of treatment (interquartile range: −0.36 to −0.04, P = 0.04) (see Figure 1; Table 1). The median reduction in urine albumin peaked at 40% (interquartile range: −0.58 to −0.15) 2 months after treatment discontinuation. Seven patients of 16 experienced >25% reduction in urine albumin. The pretreatment values ranged from 0.3 to 6.24/24 h (median: 1.6 g/24 h). Each patient’s individual change in urine albumin over time is presented in Figure 2.

The regression analyses showed a significant negative slope for urine albumin level during treatment (−1.75, −3.98 to −0.62, P = 0.02). Fourteen of 16 patients had a reduction in urine albumin over time during the treatment period. In two of these patients, the reduction in urine albumin during treatment was not larger than that observed in pretreatment, i.e. the slopes of the regression lines were steeper before than during treatment. When considering the difference in regression line coefficients before and during treatment, 12 patients may be considered as having responded to treatment. The difference in regression line coefficients (prior to and during medication) reached borderline significance (1.6, −0.16 to 4.95, P = 0.07)
due to four non-responders. The blood pressures were not changed from baseline until the end of treatment.

The reduction in serum creatinine was modest but significant (median 7.5 μmol/L, interquartile range: −15 to −1, P = 0.005), giving a relative reduction in serum creatinine of 6% (interquartile range: −0.12 to −0.02, P = 0.003). The regression analysis showed a borderline significant negative slope for creatinine during treatment (−0.04, −0.06 to −0.0002, P = 0.05). Twelve of 16 patients had a reduction in serum creatinine versus time during the treatment period, with pretreatment values between 60 and 138 μmol/L (median: 105 μmol/L). However, the difference in coefficients (prior to and during medication) was not significant.

The eGFR (MDRD) increased by ~8% (median) from baseline until the end of treatment (interquartile range: 0.02–0.16, P = 0.003). The regression analysis showed a borderline significant negative slope for creatinine during treatment (−0.003). The regression analysis showed a borderline significant negative slope for creatinine during treatment (−0.04, −0.06 to −0.0002, P = 0.05). Twelve of 16 patients had a reduction in serum creatinine versus time during the treatment period, with pretreatment values between 60 and 138 μmol/L (median: 105 μmol/L). However, the difference in coefficients (prior to and during medication) was not significant.

The eGFR (MDRD) increased by ~8% (median) from baseline until the end of treatment (interquartile range: 0.02–0.16, P = 0.003), absolute change 7 mL/min/1.73m² (interquartile range: 1–10) (see Figure 3; Table 1).

Eleven of 16 patients had an increased eGFR (MDRD) during the treatment period, with pretreatment values between 49 and 108 mL/min/1.73m² (median: 64 mL/min/1.73m²). The eGFR was increased by ~4 mL/min (median) when estimated by the Cockcroft–Gault formula (interquartile range: 0–12), relative change 4% (interquartile range: −0.01 to 0.11, P = 0.06). When using the GFR values determined from the plasma levels of cystatin C, no increase in GFR was found (see Table 1).

There were no significant differences in IgA concentrations before and after treatment. The median serum IgA concentration was 3315 μg/mL (range: 1500–5400 μg/mL) at baseline and 3450 μg/mL at end of treatment/treatment withdrawal (range: 1700–5500 μg/mL).

Measurements of HAA-IgA levels (a measure of serum Gal-deficient IgA1), both before and after removal of sialic acid by neuraminidase treatment, showed very small differences when comparing values before and after treatment for each individual patient. There was no significant difference between serum HAA-IgA levels when comparing pretreatment and post-treatment samples. The median serum HAA-IgA levels, measured as absorbance at 405 nm, were 0.29 (range: 0.17–0.43) pretreatment and 0.30 (range: 0.17–0.43) post-treatment. The corresponding median values for neuraminidase-treated samples were 0.50 (range: 0.39–0.68) and 0.49 (range: 0.39–0.68) for pretreatment and post-treatment samples, respectively. Furthermore, no changes in IgA antibodies against gliadin were found (data not shown).

Three patients were prematurely withdrawn from the study due to adverse events, of which two, both concerning abdominal pain, were considered possibly and probably related to the study drug, respectively. A third adverse event concerned sleep disturbances and increased micturition. No major corticosteroid-related side effects were observed or reported.

Two patients with baseline urine albumin levels of 260 and 460 mg/24 h were erroneously included in the study but still included in the intention-to-treat analyses. These were both classified as non-responders.

**Discussion**

In this study, we have shown that 6-month treatment with targeted release of budesonide has a significant effect on urine albumin excretion in patients with IgAN. This was accompanied by a minor reduction of serum creatinine.

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**Table 1.** Median values (interquartile range within parentheses) before treatment, after treatment and difference (with corresponding P-values for the difference) for urine albumin, serum creatinine, plasma cystatin C, eGFR (MDRD), eGFR (Cockcroft), eGFR (p-cystatin C) and blood pressure.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After treatment</th>
<th>Median of individual changes</th>
<th>Relative individual change</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-albumin (mg/24 h)</td>
<td>1579 (1086–2308)</td>
<td>1208 (1120 to 1638)</td>
<td>−529 (−60 to −757)</td>
<td>−0.23 (−0.36 to −0.04)</td>
<td>0.04</td>
</tr>
<tr>
<td>S-creatinine (μmol/L)</td>
<td>105 (94–126)</td>
<td>95 (87 to 118)</td>
<td>−7.5 (−2 to −13)</td>
<td>−0.06 (−0.12 to −0.02)</td>
<td>0.003</td>
</tr>
<tr>
<td>p-Cystatin C (mg/L)</td>
<td>1.12 (1.02–1.27)</td>
<td>0.96 (0.86 to 1.23)</td>
<td>0.01 (−0.17 to 0.10)</td>
<td>−0.02 (−0.15 to 0.08)</td>
<td>NS</td>
</tr>
<tr>
<td>eGFR (MDRD) (mL/min/1.73m²)</td>
<td>64 (54–74)</td>
<td>67 (60 to 93)</td>
<td>7 (1 to 10)</td>
<td>0.08 (0.02 to 0.16)</td>
<td>0.003</td>
</tr>
<tr>
<td>eGFR (Cockcroft) (mL/min)</td>
<td>98 (77–110)</td>
<td>97 (71 to 120)</td>
<td>4.0 (10 to 12)</td>
<td>0.04 (0.01 to 0.11)</td>
<td>0.06</td>
</tr>
<tr>
<td>eGFR (p-cystatin C) (mL/min/1.73m²)</td>
<td>74 (57–86)</td>
<td>73 (63 to 89)</td>
<td>3 (−6 to 9)</td>
<td>0.03 (−0.07 to 0.14)</td>
<td>NS</td>
</tr>
<tr>
<td>Blood pressure—systolic (mmHg)</td>
<td>123 (120–130)</td>
<td>129 (111 to 140)</td>
<td>9 (−4 to 11)</td>
<td>0.08 (−0.4 to 0.08)</td>
<td>NS</td>
</tr>
<tr>
<td>Blood pressure—diastolic (mmHg)</td>
<td>80 (77–81)</td>
<td>82 (80 to 87)</td>
<td>4 (−4 to 10)</td>
<td>0.04 (−0.04 to 0.13)</td>
<td>NS</td>
</tr>
</tbody>
</table>

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levels and a modest increase of eGFR calculated by the MDRD equation, while eGFR calculated from the Cockcroft–Gault equation and cystatin C was not changed. These results are in line with several previous clinical trials conducted with oral and intravenous corticosteroids, mostly prednisolone [4–7]. However, targeted release budesonide represents a new therapeutic concept in the treatment of IgAN in that it is a locally active glucocorticoid specifically designed to act in the ileocecal region (the Peyer’s patches).

There is increasing evidence that the mucosal immune system is involved in IgAN. The mesangial deposits of IgA in IgAN are mainly polymeric IgA1, for which the mucosal immune system is a major source. Infections in the respiratory or gastrointestinal tract with a triggered mucosal immune response are commonly associated with IgAN [12]. Several gastrointestinal antigens, in particular food antigens, have been proposed to be involved in the pathogenesis of IgAN in a subgroup of patients [13–16], and an association between celiac disease and IgAN has been reported [17]. A few studies have demonstrated deposits of food antigens in the glomeruli [14, 18], circulating antibodies against food antigens [19] and food antigens in circulating immune complexes [20]. Furthermore, increased intestinal permeability, commonly associated with gastrointestinal inflammation, has been demonstrated in IgAN [21, 22]. Such intestinal barrier dysfunction could result in loss of mucosal antigen exclusion and systemic absorption of antigens or even antigen–antibody immune complexes with subsequent deposits in the glomeruli. Taking these results into consideration, the gastrointestinal immune system may play an important part in the pathogenesis of IgAN.

The optimal therapeutic strategy in IgAN would be to identify and eliminate the gastrointestinal antigens. However, since the exact antigens involved in IgAN are usually not known and may be numerous, the alternative strategy would be to suppress the mucosal immune response to gastrointestinal antigens. This is what we have demonstrated is possible by use of targeted release budesonide. The efficacy of budesonide in this study supports the hypothesis of a mucosal immune response component being an important factor in the pathogenesis of IgAN.

As with all glucocorticoids, budesonide exerts its effect by binding to glucocorticoid receptors with subsequent up-regulation of anti-inflammatory proteins and repression of proinflammatory proteins. Due to the low systemic availability of budesonide and the major contribution of the mucosal immune system in IgAN, the effect of budesonide in IgAN is assumed to be through binding to glucocorticoid receptors in the gastrointestinal mucosa or submucosa and thus suppression of the gastrointestinal immune response. However, glucocorticoid receptors are also present on the podocytes, and an interaction with the podocytes by budesonide or its metabolites at this level cannot be excluded, as it has previously been hypothesized that the reduction of proteinuria by systemic corticosteroids is partly due to a direct effect on the podocytes [23].

Proteinuria is a major risk factor for progression of IgAN [24], and it is therefore one of the most commonly chosen surrogate end points in IgAN clinical trials. In our study, the effect of budesonide on albuminuria was modest but significant. The largest effect was seen 2 months after treatment discontinuation. The reason for the delayed effect is not clear but may be related to adaptation mechanisms, since there was an apparent lag time between initiation of treatment and onset of the effect. Interestingly, the same delayed effect was not seen with respect to serum creatinine levels nor eGFR.

The effect on urine albumin levels does not seem to be persistent in all patients, however. Three months after end
of treatment, the albumin values were increasing in several of the patients, which is contrary to what is seen with systemic corticosteroids where the effect following a 6-month course seems to last several years after end of treatment [8]. This could indicate that the mechanism of action of local corticosteroids, like budesonide, is different from that of systemic corticosteroids in IgAN.

Fourteen of 16 patients had a reduction in urine albumin during the treatment period, whereas 12 patients were defined as responders when considering the pretreatment progress. No obvious similarities were found among the non-responders. One of the patients was withdrawn from the study due to abdominal pain and did not take the full course of treatment. It is likely that truncated treatment is the reason for the lack of effect in this patient. For the other patients, it could be that other pathogenic mechanisms rather than mucosal involvement were contributory or that the patients’ renal functions were insufficiently impaired in order to be able to see any effect. A recent study by Harada et al. [25] showed that 12 of 50 patients receiving corticosteroid treatment were non-responders (defined as no reduction in proteinuria and/or progression of renal impairment) and that high numbers of fibroblast-specific protein 1-positive [FSP1(+)] cells in kidney at diagnosis correlated with steroid resistance. It is possible that the level of FSP1(+) cells is of relevance for predicting the response to budesonide as well and this is of interest to investigate in further studies.

ACE inhibitors and ARBs are known to ameliorate proteinuria in IgAN patients [1]. Fourteen of 16 patients received such treatment. However, as all antihypertensive regimens were stable during the study, this is unlikely to have influenced the study results. It should be noted that blood pressure was not altered during the study.

The eGFR increased during the treatment period when using the MDRD formula. However, this result could not be confirmed when using the Cockcroft–Gault formula or when analyzing GFR derived from plasma cystatin C. No indirect measures for kidney function are excellent. It is known that the MDRD formula is not optimal in case of GFR >60 mL/min [26], which was the case for the majority of our patients. Also, estimations based on cystatin C have limitations as cystatin C has high intrapatient variability [27], and systemic glucocorticoids might in a dose-dependent manner underestimate GFR calculations [28]. Whether targeted release corticosteroids will underestimate the plasma cystatin C-based GFR calculations has not been studied but might be of importance. Direct measurements of GFR or a longer study duration could have clarified any potential effect of budesonide on the GFR.

Increased concentrations of serum IgA and an elevation of galactose-deficient IgA1 are commonly associated with IgAN. In the present study, there was no statistical difference in serum IgA concentrations or HAA-IgA levels when comparing values before and after treatment with budesonide. When comparing the HAA-level before and after treatment for each individual, there was very little difference (correlation coefficient: 0.94). This is in agreement with recent data indicating that galactose-deficient IgA1 may be an inherited trait [29].

The limitations of our exploratory study include the relatively short duration with a treatment period of only 6 months and the use of a surrogate primary end point (urine albumin levels). As the treatment is intended for chronic administration, larger studies with longer duration must be conducted to investigate potential side effects with long-term use. No direct measurements of GFR by use of e.g. iohexol or inulin were performed, limiting the interpretation of the eGFR results. Furthermore, the study was a pilot trial including no control group. A large-scale, prospective, randomized controlled clinical trial needs to be performed in order to confirm the results of the present study.

In conclusion, local immunosuppressive treatment of the gut mucosa with targeted release of budesonide may represent a new approach to treatment of IgAN. The present 6-month study has demonstrated proof-of-concept for the use of enteric budesonide targeted to the ileoceleal region in IgAN, leading to a modest, but significant reduction in urine albumin, a minor reduction of serum creatinine and a modest increase of eGFR calculated by the MDRD equation, whereas eGFR calculated from Cockcroft–Gault equation and cystatin C was not changed.

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Conflict of interest statement. B.F. is a stockholder (<3%) in Pharmalink AB, Sweden.

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