Longitudinal relationships between fluid status, inflammation, urine volume and plasma metabolites of icodextrin in patients randomized to glucose or icodextrin for the long exchange

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Abstract

Background. Randomized trials have shown that icodextrin reduces the volume of extra-cellular fluid (ECFv) with variable effects on residual renal function. To explore this fluid shift and its possible mechanisms in more detail, prospectively collected data from one such trial, including measures of inflammation (C-reactive protein, tumour necrosis factor-α, albumin and low and high molecular weight hyaluronan) ANP (atrial naturetic peptide), an indirect marker of intra-vascular volume, plasma concentrations of icodextrin metabolites and α-amylase activity were analysed.

Methods. 50 patients were randomized to either 2.27% glucose or icodextrin (n = 28) for a long exchange following a month run in. Blood samples were obtained at −1, 0, 3 and 6 months, coincident with measurements of urine volume and fluid status.

Results. In both randomized groups, a significant correlation between the fall in ECFv and the decline in urine volume was observed (P = 0.001), although the relative drop in urine volume for patients randomized to icodextrin tended to be less. At baseline, ANP was higher in patients with proportionately more ECFv for a given body water or height. Icodextrin patients had non-significantly higher ANP levels at baseline, whereas by 3 (P = 0.026) and 6 months (P = 0.016) these differed between groups due to divergence. There was a correlation between increasing ANP and reduced ECF at 3 months, r = −0.46, P = 0.007, in patients randomized to icodextrin, but not glucose. There were no relationships between fluid status and any inflammatory markers at any point of the study, with the exception of albumin at baseline, r = −0.39, P = 0.007. Amylase activities at −1 month and baseline were highly correlated, r = 0.89, P < 0.0001. Within patients, concentrations of icodextrin metabolites were highly correlated; the only predictor of between-patient variability on multivariate analysis was body weight. There was no relationship between plasma concentrations of icodextrin metabolites and any of the other clinical parameters, including change in daily ultrafiltration, urine volume, fluid or inflammatory status.

Conclusions. This analysis supports observational data that changes in fluid status are associated with changes in urine volume. Icodextrin was not associated with a greater fall in urine output despite its larger effect on ECFv. Changes in fluid status could not be explained or did not appear to influence systemic inflammation. Nor can they be explained by individual variability in plasma concentrations of icodextrin that are in turn inversely proportional to the volume of distribution.

Keywords: amylase; bioimpedance; fluid status; inflammation; icodextrin

Introduction

Icodextrin has been shown to alter fluid status by reducing the extra-cellular fluid volume (ECFv) [1–3]. This has not always translated into associated changes in blood pressure (BP), however, and the impact on residual urine volume has also varied, with one study showing a fall, others a relative preservation of urine volume [1–4]. Possible explanations might include trial design leading to variable fluid status at baseline, differences in the incremental changes in fluid removal or good BP control at baseline leading to changes in medication during the course of the study that might
have masked any clear effect. Alternatively, the relationship between fluid removal, ECFv and urine volume may differ between all glucose regimes and those including icodextrin.

We undertook further analysis of prospectively collected data and samples from a randomized, double-blind placebo-controlled trial designed to evaluate the effects of icodextrin on fluid status in order to try and answer the following questions: (i) Was there a relationship between changing fluid status and urine volume and if so, was this the same for glucose and icodextrin? (ii) Was there indirect evidence of a change in intra-vascular volume as judged by plasma atrial natriuretic peptide (ANP), and could this be related to changing fluid status? (iii) Was there a relationship between fluid status changes and inflammatory status? (iv) Could the variability in response to icodextrin be explained by differences in the plasma concentrations of icodextrin metabolites?

Methods

Study design

A detailed description of the randomized trial has been published previously [1]. Briefly, important inclusion criteria were (a) either untreated hypertension (BP > 140/90), treated hypertension or a dialysis prescription with a daily average glucose concentration of 2.27% or greater; (b) high or high-average peritoneal solute transport (corrected 4-h D/P creatinine ratio ≥ 0.65) and (c) urine output ≤ 750 ml/day. Following a run-in period of 1 month during which subjects used glucose 2.27% in the long exchange group in the repeated measures ANOVA was not significant so whether or not it was the same for glucose and icodextrin. Multiple regression was performed where multiple correlations were observed, e.g. when determining factors associated with baseline fluid status and concentrations of plasma icodextrin metabolites. Where appropriate, data were first log transformed.

Clinical and laboratory measurements

The ECFv and total body water volume (TBWv) were determined using multiple frequency bioelectrical impedance analysis performed with the Hydra 4200 analyser (Xitron Technologies, San Diego, CA, USA). TBWv was also determined independently from deuterium (D) dilution as described. In the primary analysis, longitudinal changes in the ECFv correlated with changes in TBWv when determined from D dilution. Residual urine volume was determined from a 24-h collection.

Laboratory measurements

Plasma samples were put on ice after collection and stored at −80°C. Analyses were performed centrally. CRP was determined from a high-sensitivity assay. TNF-α was determined by immunometric assay (Immulite, DPC, Siemens, California), and both HMW- and LMW-HA were determined by high-sensitivity, proteoglycan-dependent time-resolved immunoassay [5]. ANP was extracted from plasma using Sep-pak-C18 columns and quantified with an 125I resolved immunoassay [5]. Plasma α-amylase activity was measured using an adapted routine method using p-nitrophenol maltolheptaoside as a substrate [7]. Plasma icodextrin metabolites were measured using gel filtration high-performance liquid chromatography as described [8]. The concentrations of G8 to G10 are estimated as no commercial standards are available.

Statistics

Between-group and longitudinal within-group analyses were made using unpaired and paired tests, respectively, employing parametric and non-parametric tests where appropriate and Bonferroni’s correction for multiple comparisons. Correlations between the changes in urine and ECF volumes and other measures were performed using Pearson’s or Spearman’s coefficients having first checked for linearity. A mixed-model repeated measures ANOVA (SAS) was used to investigate whether there was a relationship between changing fluid status and urine volume and if so whether or not it was the same for glucose and icodextrin. Multiple regression was performed where multiple correlations were observed, e.g. when determining factors associated with baseline fluid status and concentrations of plasma icodextrin metabolites. Where appropriate, data were first log transformed.

Results

Relationship between changes in ECFV and urine volume

Following randomization at both 3 and 6 months, there was a significant relationship between the longitudinal change in ECFv and the change in urine volume in both treatment groups. In each case, a reduction in ECFv was associated with a reduction in urine volume and vice versa (see Figure 1a and b), repeated measures ANOVA $P < 0.001$. At 3 months, for glucose the correlation was $r = 0.44$, $P = 0.037$, and for icodextrin $r = 0.5$, $P = 0.013$. At 6 months, for glucose the correlation was $r = 0.63$, $P = 0.02$, and for icodextrin $r = 0.6$, $P = 0.003$. The effect of treatment group in the repeated measures ANOVA was not significant ($P = 0.39$), although there was if anything a tendency for a greater fall in urine volume for a given change in ECFv in those treated with glucose in the long dwell (see Figure 1).
Fig. 1. Relationship between the change in ECFv and urine volume observed at (a) 3 months and (b) 6 months post-randomization. Data are shown separately for the icodextrin treated (•, solid regression line) and glucose 2.27% (□, dashed regression line). At both time points for both solutions, there was a significant negative relationship (see the text for details). The relative reduction in urine volume for a given fall in ECFv appears less in the icodextrin group.

Relationship between fluid status, fluid removal and ANP

The hydration state of patients on PD can be expressed as the ECFv/TBWv ratio, obtained from bioimpedance measurements. Using this ratio as the dependent variable, multivariate linear regression was used to identify factors that correlated with fluid status at baseline (n = 50), see Table 1. Two factors were found to have a significant relationship, peritoneal fluid removal and plasma ANP, which were decreased or increased with increasing hydration, respectively. ECFv/height, another approach to assessing fluid ECFv excess, also correlated with ANP, r = 0.52, P < 0.001. Following randomization, no such relationship was found with any of the clinical variables and hydration. Prior to the intervention, patients who were subsequently randomized to icodextrin had a non-significantly higher plasma ANP than the glucose group; following randomization, there was a divergence in the ANP concentrations such that they became significantly different, see Table 2. In the icodextrin-treated patients, the fall in ECFv correlated with an increase in ANP, r = −0.46, P = 0.007, whereas no such relationship was observed in the glucose-treated group.

CRP correlated at each time point with total HA (range of r values: 0.33–0.45, P < 0.035), due to correlations with HMW-HA only and inversely with plasma albumin (r = −0.34, P = 0.028). On initial analysis, there was a positive correlation between TNF-α and total HA at each of the time points during the study. This was due to a single outlying patient with an underlying diagnosis of systemic lupus, with a normal CRP, whose TNF-α and HA levels were an order of magnitude higher than that of the remaining patients. When this patient was excluded from further analysis, there were no between-group or within-group longitudinal differences in TNF-α during the course of the study Table (2). At each time point, there was a significant but weak negative correlation between LMW-HA and urine volume, with r values ranging between −0.2 and −0.37, P < 0.05, but not with HMW-HA. Within each group total, LMW- and HMW-HA increased with time during the study; this was more significant in the icodextrin-treated group, especially for HMW-HA, but this difference was already seen in the month prior to randomization. There were no relationships observed between fluid status (either ECFv/TBW or ECFv/height) and inflammatory markers, with the exception of plasma albumin at baseline on univariate analysis (r = −0.39, P = 0.007), although this disappeared on multivariate analysis (Table 1).

Table 1. Multivariate analysis of covariates associated with fluid status at baseline (dependent variable ECFv:TBW ratio from bioimpedance)

<table>
<thead>
<tr>
<th>Covariates</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>2.0</td>
<td>0.053</td>
</tr>
<tr>
<td>Plasma albumin (g/l)</td>
<td>−1.11</td>
<td>0.28</td>
</tr>
<tr>
<td>Atrial natriuretic peptide</td>
<td>2.69</td>
<td>0.011</td>
</tr>
<tr>
<td>Urine volume (ml)</td>
<td>−1.74</td>
<td>0.091</td>
</tr>
<tr>
<td>Net peritoneal ultrafiltration (ml)</td>
<td>−2.6</td>
<td>0.014</td>
</tr>
</tbody>
</table>
Longitudinal relationships between fluid status, inflammation, urine volume and plasma metabolites

Table 2. Longitudinal changes in ANP, inflammatory markers, amylase activity, total icodextrin metabolites and osmolality according to the treatment group

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Study timepoint</th>
<th>−1 month</th>
<th>Baseline</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrial natriuretic peptide (pg/ml)</td>
<td>Icodextrin</td>
<td>324 (38)</td>
<td>317 (34)</td>
<td>368 (45)</td>
<td>364 (47)</td>
</tr>
<tr>
<td>Glucose</td>
<td>239 (44)</td>
<td>236 (57)</td>
<td>220 (44)</td>
<td>186 (49)</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (median, range) (mg/l)</td>
<td>Icodextrin</td>
<td>6.0 (40)</td>
<td>3.0 (42)</td>
<td>5.0 (115)</td>
<td>4.5 (179)</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.5 (40)</td>
<td>4.0 (54)</td>
<td>7.0 (45)</td>
<td>6.0 (23)</td>
<td></td>
</tr>
<tr>
<td>Tumour necrosis factor (pg/ml)</td>
<td>Icodextrin</td>
<td>11 (0.8)</td>
<td>10.5 (0.8)</td>
<td>11.1 (0.7)</td>
<td>11.5 (0.6)</td>
</tr>
<tr>
<td>Glucose</td>
<td>12.7 (0.9)</td>
<td>12.7 (1.3)</td>
<td>13.4 (1.2)</td>
<td>12.8 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Total hyaluronan (µg/l)</td>
<td>Icodextrin</td>
<td>125 (7.4)</td>
<td>148 (10)</td>
<td>207 (19)</td>
<td>223 (29)</td>
</tr>
<tr>
<td>Glucose</td>
<td>158 (13.5)</td>
<td>171 (15)</td>
<td>238 (67)</td>
<td>178 (15.6)</td>
<td></td>
</tr>
<tr>
<td>HMW hyaluronan (µg/l)</td>
<td>Icodextrin</td>
<td>101 (7)</td>
<td>123 (11.3)</td>
<td>176 (18.2)</td>
<td>193 (28)</td>
</tr>
<tr>
<td>Glucose</td>
<td>132 (11.6)</td>
<td>142 (46)</td>
<td>209 (66)</td>
<td>149 (13.9)</td>
<td></td>
</tr>
<tr>
<td>LMW hyaluronan (µg/l)</td>
<td>Icodextrin</td>
<td>24.6 (1.6)</td>
<td>25.8 (1.3)</td>
<td>30.7 (2.5)</td>
<td>30.3 (2.8)</td>
</tr>
<tr>
<td>Glucose</td>
<td>25.9 (2.6)</td>
<td>28.9 (5)</td>
<td>29.3 (2.7)</td>
<td>28.6 (2.5)</td>
<td></td>
</tr>
<tr>
<td>Amylase activity (U/l)</td>
<td>Icodextrin</td>
<td>83 (6.7)</td>
<td>89 (7.6)</td>
<td>18 (2.5)</td>
<td>16 (2.6)</td>
</tr>
<tr>
<td>Glucose</td>
<td>78 (8.6)</td>
<td>77 (10.2)</td>
<td>73 (10.4)</td>
<td>80 (10.3)</td>
<td></td>
</tr>
<tr>
<td>Total icodextrin metabolites (mg/ml)</td>
<td>Icodextrin</td>
<td>–</td>
<td>–</td>
<td>4.5 (0.28)</td>
<td>4.36 (0.39)</td>
</tr>
<tr>
<td>Glucose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Plasma osmolality (mOsm/l)</td>
<td>Icodextrin</td>
<td>298 (4.3)</td>
<td>300 (4)</td>
<td>298 (4.1)</td>
<td>298 (4.1)</td>
</tr>
<tr>
<td>Glucose</td>
<td>298 (5)</td>
<td>286 (6.6)</td>
<td>290 (5.4)</td>
<td>290 (5.4)</td>
<td></td>
</tr>
</tbody>
</table>

*Between-group difference, \( P < 0.05 \).
*bBetween-group difference, \( P < 0.001 \).
^Longitudinal difference between −1 month and baseline, \( P = 0.003 \).
^dLongitudinal difference between baseline and 6 months, \( P < 0.001 \).
^eLongitudinal difference between −1 month and baseline, \( P = 0.008 \).
^fLongitudinal difference between baseline and 6 months, \( P = 0.002 \).
^gLongitudinal difference between baseline and 6 months, \( P = 0.054 \).

**Relationship between changes in fluid status, plasma amylase activity and concentrations of icodextrin metabolites**

Prior to randomization, there was a wide range in plasma amylase activity observed (mean 80, range 35–200 U/l) with apparent stability within individuals as evidenced by a high degree of correlation between −1 month and baseline (\( r = 0.88, P < 0.001 \)) (Figure 2). There was no relationship between plasma amylase and weight, BSA, age or urine volume on log-transformed linear regression. Following randomization there was, as expected, a very significant and sustained fall in plasma amylase in patients using icodextrin (see Table 2). At 3 and 6 months following commencement of icodextrin, plasma metabolites ranging from G2 (maltose) through to G10 were detected in decreasing concentrations with an increasing molecular weight (see Figure 3). A wide range of variability between subjects was observed, but the concentrations of the metabolites correlated significantly with each other. Factors associated with plasma icodextrin metabolite concentrations were weight and to a lesser extent urine volume (Table 3). No correlations with height, gender or amylase concentrations either before or after randomization were observed. There were no correlations observed between plasma icodextrin metabolites and any of the observed changes in weight, ultrafiltration, sodium removal,
for this would be that the change in ECFv is achieved whilst protecting the patient from intravascular volume contraction. Assessing volume status in PD patients is undoubtedly difficult. There is considerable evidence that they are frequently volume loaded when compared to either normal subjects or haemodialysis patients [6], but it is less clear whether this extra fluid is predominantly in the intravascular or extracellular, interstitial space. At baseline in this study, there was an association between increased ANP levels and ECFv excess, whether expressed as a ratio of the TBW or as a function of height that could not be explained by variations in residual renal function. ANP may not be an ideal measure of intravascular volume in patients with renal failure, although there is evidence to suggest it is an indirect indicator of atrial filling in patients without serious cardiac problems [13, 14]. N-terminal brain natriuretic peptide, which was not measured in this study for technical reasons, has recently been shown to predict cardiac failure in PD patients [15]; however this might be more a marker of structural cardiac injury than fluid status in this context. The observation that if anything ANP levels tended to increase, especially in patients who had the greatest reduction in their ECFv was, therefore, surprising, but raises the possibility that there is a relative preservation of intravascular volume when using icodextrin explained by osmotic or oncocytic pressures induced by icodextrin metabolites. The present study however would not suggest that this is a function of plasma osmolality or between-patient differences in LMW icodextrin metabolites. Low concentrations of HMW icodextrin in plasma could potentially influence oncotic pressures and are difficult to measure but it must be remembered that starch derivatives are routinely used as plasma expanders. This hypothesis requires testing, but the recent report of increasing natriuretic peptides with icodextrin use in diabetic patients undergoing CAPD shows that this is not an isolated observation [16].

Previous studies have indicated that increased systemic inflammation is associated with an expanded ECFv in PD patients [17, 18]. There was some evidence for such an association at baseline in this study, but not longitudinally. This might reflect either a selection bias of relatively healthy patients or a lack of statistical power. Another weakness of the study was the failure to measure interleukin-6. The multivariate analysis disguises the fact that a higher CRP and lower albumin are associated with increasing age and both of these factors can be linked to expanded ECFv both here and in previous studies [6, 19, 20]. Previous reports of the changes in systemic inflammatory markers when using icodextrin have been variable [21, 22], but the present study did not find any between-group differences or within-group changes in either CRP, in agreement with the only
other study randomizing patients to a fluid status intervention (2), or TNF-α. The lack of a significant change in fluid status leading to an improvement in inflammatory markers is against a causal relationship in this direction. At first sight it appears that the icodextrin group tended to have a more marked longitudinal increase in plasma HA concentrations compared to the glucose group. However, this difference was already present in the month before randomization, indicating that this was a pre-existing feature of these patients. Furthermore, there were no between-group differences at any of the study time points. In view of this observation we chose to measure the high and low molecular weight components of HA. Previous studies in normal individuals have shown that daily turnover of HA, 95% of which resides in the interstitial space, is approximately one-third of its total mass [23]. Increased turnover, and thus plasma levels, may occur in response to inflammatory stimulus, such as increased TNF-α, or due to physical propulsion associated with fluid movement from the extracellular into the intravascular space, e.g. after exercise or feeding [24,25]. The pathway and fate of HA depends on its molecular size, with HMW-HA being transported by the lymphatics and metabolized by the reticulo-endothelial system, and LMW-HA excreted by the kidneys [23]. This explains the relationship observed in this study between LMW-HA levels and residual urine volume.

The only important predictor of plasma icodextrin metabolite concentrations identified here was the weight, or more precisely the volume of distribution. This is in keeping with single-exchange kinetics in which the peak plasma concentration was closely correlated to weight [26]. The failure to find any correlation with the plasma amylase activity, either before or after commencing icodextrin, would suggest that the between-patient variability in amylase—which is significant and relatively stable—is not contributing to the between-patient differences in icodextrin metabolite concentrations. This is a little surprising as amylase is considered to be the principal enzyme responsible for cleaving icodextrin metabolites and may suggest that this process occurs in the peritoneal interstitial tissues where there is tissue-bound amylase and not just the plasma compartment. The marked reduction in plasma amylase activity following commencement of icodextrin has been described previously; initially thought to be an artefact due to the interference by metabolites of the amylase assay, this has been shown not to be the case with the method used here [7].

This study found no relationship between the efficacy of icodextrin in changing ultrafiltration, fluid status or the relative preservation of residual urine volume with the concentration of plasma icodextrin metabolites. This is in keeping with previous observations that there is no relationship between metabolite concentrations and net ultrafiltration achieved with icodextrin [27].

In summary, this analysis provides further evidence for the important relationship between ultrafiltration, fluid status and preservation of urine volume in PD patients. Existing as well as future guidelines should take this into account. Icodextrin has the potential to reduce ECFv without endangering the urine volume provided care is taken to avoid excessive fluid depletion. Inflammation appears to have some role in determining fluid status but there is no clear evidence for a reverse causal relationship.

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Conflict of interest statement. B.L., A.T. and J.C.D.F are employees of Baxter Healthcare. The data have been published in part previously in an abstract form only and presented at the 2004 meeting of the International Society of Peritoneal Dialysis—EuroPD in Amsterdam, 2005 EuroPD meeting in Prague and the ASN in Philadelphia. S.J.D has received research funding and acts in an advisory capacity to Baxter Healthcare. Particular thanks go to Clive Richards and Louise Phillips for their excellent logistic support. High and low molecular weight hyaluronan fractions were analysed by Andreas Rössler (University of Graz, Austria). Tony Qureshi (Karolinska Institutet, Sweden) undertook the mixed-model repeated measures analysis.

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