Dialysis with icodextrin interferes with measurement of serum α-amylase activity

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Abstract

Background. The glucose polymer icodextrin has gained a widespread use in peritoneal dialysis especially in patients with low ultrafiltration and high peritoneal transport properties. In patients using a once-daily exchange with icodextrin, a decreased serum amylase activity has been reported. We explored the potential underlying mechanisms of this effect.

Methods. Using standard chromolytic methods, serum amylase activity was measured in blood samples from 11 patients on icodextrin treatment and from 11 patients on conventional glucose treatment. Samples were additionally supplemented with α-amylase and unused icodextrin dialysis fluid. Potential complex formation between icodextrin and α-amylase was studied by SDS-gel electrophoresis with protein silver staining and fluorophore-assisted carbohydrate staining with the AMAC (FACE) method, which provides oligosaccharide labelling. Lipase activity was measured in parallel in all samples by two standard methods.

Results. Amylase activity was reduced by 90% in serum from patients using icodextrin for the long dwell (15.9 ± 10.9 U/l) vs patients using standard glucose (157.1 ± 23.7 U/l; \(P<0.001\)). Addition of icodextrin to serum samples from patients using conventional glucose solutions induced a dose-dependent decrease in amylase activity. The assay results indicated a substrate competition between ET7-G7PNP and icodextrin dialysis fluid. Potential complex formation between icodextrin and α-amylase was studied by SDS-gel electrophoresis with protein silver staining and fluorophore-assisted carbohydrate staining with the AMAC (FACE) method, which provides oligosaccharide labelling. Lipase activity was measured in parallel in all samples by two standard methods.

Lipase measurement should provide an alternative and unconfounded method for diagnosing pancreatitis in icodextrin patients.

Keywords: α-amylase activity; icodextrin; interaction; lipase activity; pancreatitis; peritoneal dialysis

Introduction

The employment of glucose polymer (icodextrin) as an osmotic agent in peritoneal dialysis (PD) has gained widespread use during recent years. During a long dwell, icodextrin enhances ultrafiltration and small-solute clearances compared to 2.27% glucose, and thereby also avoids exposure of the peritoneum to hypertonic glucose solutions [1,2]. It is now established as an important option for treating ultrafiltration failure and overhydration. Icodextrin shows very little diffusion across the peritoneal membrane because its large molecular weight of between 1638 and 45 000 Daltons (average 16 800 Daltons). However, icodextrin and its hydrolysed metabolites (maltose, maltotriose etc.) have been found in the systemic circulation in measurable amounts mainly because of lymphatic transport from the peritoneal compartment. Plum et al. [3] reported a total serum icodextrin plus metabolite concentration of about 5.2 mg/ml (besides maltose) after 6 weeks of icodextrin treatment. In 1998, icodextrin was reported to interfere with a specific enzymatic glucose measurement method [4,5]. This produced an overestimation of blood glucose in icodextrin-treated patients tested with glucose dehydrogenase, an enzyme of the pyrroloquinoline quinone class reacting with the free reducing group of the glucose molecule located at the end of each saccharide strain (e.g. used by the Accutrend Sensor®).

In an ongoing multicentre study, we measured serum α-amylase activity levels. When changing from a PD regime to a once-daily exchange of icodextrin a significant drop in amylase enzyme activity was
Table 1. Clinical characteristics of glucose- and icodextrin-treated patients. All patients were free from any signs of inflammation or intestinal disease

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose group</th>
<th>Icodextrin group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42.6 ± 9.4</td>
<td>42.5 ± 25.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.5 ± 12.0</td>
<td>78.9 ± 10.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.7 ± 10.4</td>
<td>176.6 ± 10.1</td>
</tr>
<tr>
<td>Sex (female:male)</td>
<td>5:6</td>
<td>3:8</td>
</tr>
<tr>
<td>Duration of PD (months)</td>
<td>44.8 ± 52.2</td>
<td>42.1 ± 19.6</td>
</tr>
<tr>
<td>PD regime (APD-CAPD)</td>
<td>5:6</td>
<td>8:3</td>
</tr>
<tr>
<td>Icodextrin prescription (months)</td>
<td>–</td>
<td>10.5 ± 9.9</td>
</tr>
<tr>
<td>Icodextrin fill volume (l)</td>
<td>–</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

observed (after 12 weeks of treatment: glucose group: 95.2 U/l, icodextrin group: 15.6 U/l) [3]. However, a systematic investigation of the underlying mechanisms of this effect has not so far been published. There are two probable mechanisms: firstly, icodextrin may reduce pancreatic amylase production, or secondly, icodextrin may modulate amylase enzyme activity. In the present study, we intended to clarify whether icodextrin forms a complex with amylase (thereby blocking assay substrate activity) or whether the assay substrate and icodextrin act competitively as substrate for amylase. We evaluated serum samples from 22 stable patients on PD who were given either glucose or icodextrin for the long dwell.

Subjects and methods

Blood samples were drawn from 11 patients given a once-daily exchange with icodextrin for the long dwell. Blood samples from 11 PD patients given only glucose solution served as controls. Blood samples were drawn in the morning in the outpatient clinic (Vacutainer plus SST tubes coated with silicone, Beckton Dickinson, NJ, USA). All patients were free from clinical signs of infection or pancreatitis and were selected chronologically according to the date of a routine visit in the outpatient clinic (see Table 1 for patients’ characteristics).

Amylase activity was measured with the Sigma Kit No. 577 (Sigma Diagnostics, St Louis, USA). This test is based on the enzymatic cleavage of 4,6-p-nitrophenyl-z-o-maltoheptaosidethan (ET-G7PNP 1 mmol/l) by amylase to G2-, G3- and G4-PNP-fragments. Glucosidase hydrolys the G2- and G3-PNP fragments to glucose and p-nitrophenol. After a 2 min incubation period, the increase in absorbance of p-nitrophenol at 405 nm is directly proportional to the amylase activity. This system is widespread and a method of choice in clinical practice [6]. Samples were additionally supplemented with z-amylase (No. 1.16312.0001; Merck, Darmstadt, Germany) or icodextrin taken from unused Extraneal® dialysis fluid (Baxter Healthcare SA, County Mayo, Ireland). Pure standard z-amylase activities ranging from 250 to 2000 U/l were added to blood samples from patients using glucose or icodextrin dialysis fluid. In a second set of experiments, icodextrin was added to samples from patients using glucose dialysis fluid only. The icodextrin concentration in the samples was adjusted to 0.075–3.75 mg/ml, thereby approximating concentrations usually found in the serum of patients.

In additional experiments, blood samples from patients given icodextrin dialysis fluid were diluted with assay buffer. Because of the lower limitation of the test-assay, we spiked the samples with 250 U/l amylase activity before diluting. The amylase activities in the diluted samples were then recalculated with the dilution factor. For example, the amylase activity in a 4-fold diluted sample was multiplied by four.

Lipase activity was measured using a colorimetric assay (Roche Diagnostics, Indianapolis, USA) and by a turbidimetric method (Nobilflow Lipase, Nobis Labordiagnostica GmbH, Endingen, Germany).

Calibration standards were used from both manufacturers for each method. The results from the colorimetric assay were recalculated to match the results from the turbidimetric method (y = 3.4 × x − 10, where x = result in the colorimetric assay, y = recalculated result comparable to the values from the turbidimetric method). This is a standard adjustment procedure to provide comparable results from the colorimetric and turbidimetric methods (normal range 0–190 U/l at 37°C).

To detect a potential icodextrin/amylase complex formation, a polysaccharide labelling with AMAC (2-aminoacridone) followed by SDS electrophoresis was performed. AMAC is a non-charged fluorescent dye that specifically binds to polysaccharides [7,8]. Labelled icodextrin molecules are uncharged. When there is no binding to a charged component (e.g. to the negatively charged amylase in SDS), there should be no electrophoretic movement on the gel.

AMAC labelling of polysaccharides was performed as previously described [7,8]. Briefly, 10 µl samples were air dried (Techne Dri-Block, DB-3, Techne, Cambridge, UK); 5 µl of AMAC solution (A-6289, Molecular Probes, Eugene, USA) was added to samples having 5 µl 1.0 M-NaBHCN solution. The reaction mixture was incubated for 16 h at 37°C and then air dried for 2 h. Samples were dissolved in electrophoresis sample buffers and then polyacrylamide gel electrophoreses was performed (ExcelGel™ SDS, Homogeneous 12,5, Amersham Pharmacia Biotech, Uppsala Sweden, 600 V, 30 mA, 7 h). Gels were photographed under UV light (360 nm). In addition, silver staining (Plusone Silver Staining, Pharmacia Biotech, USA) was used to detect the z-amylase bands. z-glucuronic acid (G8645, Sigma, St Louis, USA), a negatively charged monosaccharide, served as an internal control.

Statistics

All data are expressed as means ± SD and were analysed with the Student’s t-test. An alpha error less than 0.05 was considered to be significant.

Results

Clinical findings in patients with pancreatitis

Three serum samples taken from patients given icodextrin during acute-phase pancreatitis had normal serum amylase activities of 87 ± 87 U/l (normal range <120 U/l). However, lipase activity was markedly increased to 1303 ± 495 U/l (normal range <190 U/l).
Amylase activity in glucose- and icodextrin-treated patients

Serum amylase activity was significantly lower in patients (free from clinical signs of pancreatitis) given once-daily exchange of icodextrin than in patients given glucose (15.9 ± 10.9 U/l vs 157.1 ± 23.7 U/l; P < 0.001, Figure 1). The addition of 250 U/l α-amylase activity to serum samples from icodextrin patients caused significantly lower recovery in these specimens than expected. In glucose-treated patients, the addition of α-amylase yielded the expected recoveries (33.3 ± 18.4 U/l in icodextrin-containing serum and 411.2 ± 24.6 U/l in icodextrin-free serum; recovery 12.3 ± 6.1 vs 101.0 ± 0.6%, P < 0.001). The external addition of 1000 U/l α-amylase activity also resulted in a significantly lower recovery in icodextrin specimens compared with the glucose samples (678.3 ± 49.4 vs 1155.7 ± 25.1 U/l; recovery 66.7 ± 4.6 vs 99.9 ± 0.2%, P < 0.001). The addition of high amylase activity (1500–2000 U/l) also resulted in reduced recovery in icodextrin patients (1500 U/l, 1156 ± 7 U/l; 2000 U/l, 1653 ± 14 U/l; recovery 77.1 ± 0.1 and 82.6 ± 0.1%).

Amylase activity was also measured in serum samples from icodextrin-treated patients after diluting the samples with the assay buffer. After a 2-fold dilution of samples from icodextrin patients and subsequent multiplication by two, we found that amylase activity increased by 26 ± 11% (P < 0.0001) compared with undiluted samples. After a 4-fold dilution, amylase activity increased by 118 ± 18% (P < 0.0001). An 8-fold dilution yielded an increase of 213 ± 37% (P < 0.0001). These results are indicative of less competitive inhibition by diluted icodextrin in these samples (while the assay substrate ET-G7PNP remained unchanged).

Icodextrin concentration-dependent reduction of α-amylase activity

Pure icodextrin solutions were added to serum samples from eight patients in the glucose group. There was a dose-dependent decrease in α-amylase activity (Figure 2). At an icodextrin concentration of 3.75 mg/ml, which approximates the lower range of typical icodextrin concentrations in serum, amylase activity was decreased by 35.1 ± 5.5% (P < 0.001). When icodextrin was spiked from 0.075 to 3.75 mg/ml in samples from patients given glucose dialysis fluid, a 50% reduction in amylase activity was calculated for an icodextrin serum concentration of 1.75 mg/ml (y = 9.07x2–60.60x + 153.98, where x = icodextrin concentration, and y = amylase concentration).

Lipase activity

Lipase activity measured by the colorimetric method was 123 ± 74 U/l in the control group and 285 ± 117 U/l in patients using icodextrin solution for the long dwell. When assessed by the turbidimetric method, the groups were not significantly different (115 ± 72 U/l in the control group and 285 ± 117 U/l in the icodextrin group). After adding varying concentrations of icodextrin to serum samples from control patients, lipase activity was not significantly altered.
Fig. 3. Upper panel: SDS gel electrophoresis of α-amylase and icodextrin-α-amylase mixture visualized by silver staining. Lower panel: fluorophore assisted carbohydrate electrophoresis (FACE) with AMAC labelled icodextrin and α-amylase. Negatively charged glucuronic acid (0.5 μg) served as a control (lane 3). No electrophoretic shift of AMAC-labelled icodextrin was observed, indicating that icodextrin did not form a complex with α-amylase.

(no icodextrin, 95 ± 55 U/l; 0.375 mg/ml icodextrin, 95 ± 56 U/l; 3.75 mg/ml icodextrin, 99 ± 56 U/l).

AMAC labelling of icodextrin

AMAC labelling of icodextrin and subsequent polyacrylamide gel electrophoresis showed clear staining of icodextrin and no electrophoretic mobility which is expected from these uncharged molecules. The negatively charged glucuronic acid used as an internal control showed the expected staining and molecular size-dependent mobility with a localization near the electrophoretic front. Co-incubation of AMAC-labelled icodextrin with α-amylase at 37°C for 1 h and subsequent electrophoresis did not produce a mobility shift of icodextrin from the starting point. There was no fluorescence on the SDS gel at the location where α-amylase was detected by silver staining (Figure 3).

Discussion

During more than 10 years of icodextrin use in the clinic, there have been no reports of histomorphological alterations in the pancreas, higher incidences of pancreatitis or changes in blood sugar control. There have, however, been reports of low amylase activity, although little clinical relevance was attached to these [9]. In three patients with clinical signs of pancreatitis we found amylase levels in the normal range and significantly elevated lipase activity.

The hypothesis of this study was that the low amylase activity measured in PD patients using icodextrin could be due to interference of icodextrin with the assay system or to an inhibition of amylase activity by complex formation.

We found moderately elevated amylase levels in dialysis patients using glucose solutions and elevated lipase concentrations in both groups. This is in accordance with many other studies and is due to impaired clearance levels in patients with renal insufficiency [10]. Furthermore, there was a dose-dependent decrease in amylase activity after adding icodextrin to serum samples from patients using glucose PD solutions. This may be explained by competitive assay interaction or by a complex formation between icodextrin and amylase. The clear dose-dependent decrease in α-amylase activity in the standard assay system points to a competitive antagonism. After diluting the serum samples from icodextrin-treated patients, an increase in measured amylase activity was found. This also indicates a competitive antagonism in the test assay. Because the labelled oligosaccharide, ET-G7PNP, is similar in structure to the 1–4 linked polyglucose molecule, icodextrin, a competitive interaction between the two substrates is probable. Although the Km value of soluble starch is 0.3–2.5 mg/ml, this value is not known for icodextrin. The ratio of labelled substrate to icodextrin was approximately 10:1 in the assay when using serum from patients given 7.5% icodextrin once daily that produced a 5.2 mg/ml serum concentration of icodextrin plus its metabolites, except for maltose, which is not cleaved by amylase [3]. This value per se does not indicate a clear substrate excess for icodextrin which would be expected for >90% reductions in enzyme activity from the assay. However, we speculate that amylase affinity for icodextrin may be higher than that of the labelled test oligosaccharide in the assay. Icodextrin is a β-glucose polymer consisting mainly of α-1,4-linkages. Amylase breaks α-1,4-linkages. Since most methods for determining amylase in routine clinical practice are based on the consumption of substrate, spuriously low concentrations of serum amylase may be measured. In addition, icodextrin has a small portion of 1,6-linkages that may hamper amylase activity.

To exclude the possibility that amylase activity was inhibited by a fixed complex formation, we performed experiments with the fluorescent dye AMAC, which was covalently coupled to icodextrin. It was then co-incubated with α-amylase and studied by SDS gel electrophoresis. If icodextrin and α-amylase AMAC form a stable complex, fluorescence should be detected at the α-amylase location on the SDS gel. Neither AMAC labelling of pure icodextrin nor combinations of icodextrin and α-amylase demonstrated any shift of α-amylase movement on the gel (silver staining). Additionally, there was no AMAC fluorescence near the region of the α-amylase spot. Since AMAC-icodextrin is a non-charged molecule, these data provide no evidence for an irreversible complex formation between icodextrin and α-amylase.

Previous studies examined potential decreases in amylase release from the pancreas by utilizing radioimmunoassay [11]. With this method, the total protein mass of amylase is measured instead of the enzyme activity. A low concentration would then be indicative of a reduced pancreatic amylase release into the blood instead of a change in its activity. Although there is little information on this method, one study indicated that radioimmunoassay values were unchanged [11].
These data also support the assumption that icodextrin does not inhibit amylase release.

Lipase activity was not altered in patients using icodextrin. Exogenous addition of icodextrin to serum samples also had no significant effect on lipase activity. Two independent methods (colorimetric assay and turbidimetry) were used in this examination. A similar finding has also been described by Grzegorzewska et al. [11]. Contrary to α-amylase, lipase activity closely corresponded to the clinical signs of pancreatitis.

Therefore, lipase determination should be the biochemical method of choice to confirm the diagnosis of pancreatitis in PD patients on treatment with icodextrin.

References


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