Heat sterilization of peritoneal dialysis solutions influences ingestive behavior in non-uremic rats

ZH-HUA ZHENG, BJORN ANDERSTAM, ABDUL RASHID QURESHI, OLOF HEIMBURGER, TAO WANG, PER SODERSTEN, JONAS BERGSTROM, and BENGT LINDHOLM

Divisions of Baxter Novum and Renal Medicine, Karolinska Institutet, Huddinge University Hospital, Stockholm, Sweden; Department of Nephrology, The First Affiliated Hospital, Sun Yat-Sen University of Medical Science, Guangzhou, P.R. China; and Section of Applied Neuroendocrinology, Karolinska Institutet, Novum, Stockholm, Sweden

Heat sterilization of peritoneal dialysis solutions influences ingestive behavior in non-uremic rats

Background. The appetite inhibitory effect of glucose-based peritoneal dialysis (PD) solutions may be due to glucose as such, or the hyperosmolality of the PD solution, or an effect of glucose degradation products (GDPs) formed in the PD solution during heat sterilization. This was studied in an experimental appetite model in rat.

Methods. The effect of different experimental PD solutions on ingestive behavior was investigated in non-uremic rats equipped with an implanted intraoral (i.o.) cannula through which a 1 mol/L sucrose solution was infused during tests. The amount of intake was recorded at 30 min after rats were infused intraperitoneally (IP) with 30 mL of different solutions. This method allowed an accurate and reproducible analysis of i.o. intake. The experimental PD solutions tested included (1) glucose based PD solutions with different glucose concentrations, sterilized by heat or microbiological filter, (2) glucose- and manitol-based PD solutions with the same osmolality, sterilized by heat or microbiological filter; and (3) glucose based PD solutions, using different pH values (pH 3.0, pH 5.5 or pH 7.4) during heat sterilization.

Results. Following IP infusion of solutions, (1) the i.o. intake was significantly inhibited by glucose based, heat sterilized PD solutions and the degree of appetite suppression was related to the concentration of dialysate glucose in a dose-dependent way; (2) the i.o. intake was significantly less suppressed by filter sterilized than by heat sterilized glucose-based solutions; (3) the i.o. intake was significantly less following the IP infusion of glucose-based than following the manitol-based heat sterilized solutions; however, i.o. intake did not differ between the glucose-based and manitol-based filter sterilized solutions; and (4) furthermore, the degree of suppression of i.o. intake induced by glucose-based PD solutions was influenced by the pH value during heat sterilization. The lower the pH of the PD solution during heat sterilization, the higher the i.o. intake.

Conclusions. The IP infusion of glucose-based heat-sterilized PD solutions inhibited food intake in this experimental appetite model, and the degree of suppression depended on the concentration of dialysate glucose and the pH of the solution during heat sterilization. The results suggest that GDPs formed during heat sterilization may exert a more adverse effect than glucose itself on ingestive behavior, and that a reduction of the concentration of GDPs in the PD solution using filter sterilization or a low pH value in the PD solution during heat sterilization may improve food intake.

A large proportion of patients treated with continuous ambulatory peritoneal dialysis (CAPD) are malnourished [1–3]. Inadequate food intake and anorexia are contributing factors, and this may be in part due to uremia and persistent uremic toxicity due to under-dialysis [1, 4]. However, loss of appetite also may be secondary and due to abdominal distension by the dialysate and absorption of glucose from the dialysate [1, 5]. Thus, the conventional glucose-based heat-sterilized peritoneal dialysis solutions, with a high concentration of glucose (13.6 to 38.6 g/L), may contribute to anorexia. In chronic peritoneal dialysis, the patients may be exposed to 50 to 100 kg of glucose per year. Glucose is not an inert osmotic agent but a nutrient that can contribute to as much as one third of the total energy intake, especially in CAPD patients with high peritoneal permeability [2, 6]. Likewise, glucose-based peritoneal dialysis (PD) solutions may contribute to hyperglycemia, hyperinsulinemia, hyperlipidemia and obesity [7]. In addition, other factors in the PD solutions such as pH, and type and concentration of buffer (in commercial solution usually 35 to 40 mmol/L of lactate) also may contribute to hypophagia.

Increasing evidence from several in vitro and in vivo studies suggests that glucose degradation products (GDPs) are formed in peritoneal dialysis fluids during heat sterilization and storage and contribute to the cytotoxicity and bioincompatibility of PD solutions [8–10]. The accumulation of GDPs in the dialysis fluid seen as a browning of the fluid can be visualized by ultraviolet absorbance and can be quantified by high performance liquid chromatography (HPLC) analysis [11, 12]. GDPs are small molecular weight products, and include aldehydes and...
other compounds such as methylglyoxal and 3-desoxyglucosone; however, the full spectrum of various GDPs generated during heat sterilization of glucose-based PD solutions is still not known [11, 12]. Glucose degradation is reduced at low pH during heat sterilization and during storage of PD solutions, and is enhanced in the presence of catalyzing substances such as lactate, calcium, and magnesium [13, 14]. GDPs in PD fluids may have many adverse effects such as abdominal pain or discomfort during infusion, inhibition of cell proliferation, inflammation of inflammatory cell function, cytotoxicity, and promotion of the formation of advanced glycation end products (AGEs) in the peritoneum [8, 15, 16]. However, the possible effect of GDPs on appetite has not been studied previously.

In an experimental appetite model in the rat, we have previously shown that the high lactate and glucose concentration in the PD solutions may inhibit appetite apparently more than pH and volume of the infused solution during PD [17–19]. However, the possible separate effects of glucose in PD solution on appetite caused by hypertonicity, absorption of glucose, and effects of breakdown products of glucose were not analyzed in previous studies. The present study used an experimental appetite model in the rat to investigate the impact on appetite of (1) dialysate glucose concentration, (2) dialysate hypertonicity, (3) heat sterilization of PD solutions, and (4) pH in PD solutions during heat sterilization.

**METHODS**

**Experimental appetite model**

Male Wistar rats (Möllegård Breeding Laboratories, Ejby, Denmark) weighing 310 to 330 g and having free access to water and pellets, were maintained in individual cages in air conditioned, temperature controlled 22°C colony rooms in which the lights were off between noon and midnight. Since darkness is an important factor for the normal ingestive behavior of the rat, the rats were tested (see below) during this period. The methods have been described in detail previously [20].

Briefly, all surgery was performed under pentobarbital anesthesia (60 mg/kg IP; Mebumal; Nordic, Stockholm, Sweden). The rat was placed in a stereotaxic frame and an incision was made in the midline of the scalp. Three screws were fixed in the skull to maintain the intraoral tube. A 5 cm long PE-100 tubing was then placed between the cheek and gum at a point slightly anterolateral to the first upper molar on the side of the mouth. The tubing was advanced subcutaneously with the distal end of the tubing protruding above the surface of the skull, while a 9 mm piece of stainless steel tubing was inserted within the roof of the mouth, between the cheek and gum. Dental cement was used to cover and fix the area between the screws and cannula. The animals were allowed three weeks of recovery after surgery. The Animal Ethical Committee of Karolinska Institutet at Huddinge University Hospital approved the study.

**Measurement of ingestive behavior**

Prior to the formal experiments, rats were given a series of training for two weeks initially until the rate of intraoral (i.o.) intake had stabilized [19, 20]. Pellets were removed at 07.00 hours and replaced after testing. Animals were tested for sucrose ingestion at 13.00 hours (one hour after the strong lights were switched off, but using indirect weak light for observation of the test). During the test, the animals were placed in a circular (35 cm diameter) Plexiglas™ arena and had their i.o. cannula connected to a peristaltic pump (Alitea xv; Ventur Alitea, Stockholm, Sweden), which delivered 1 mol/L of sucrose solution at an infusion rate of 1 mL/min. The duration of i.o. intake ended by the time the animal passively let the solution drip from its mouth. The i.o. infusion was interrupted for 30 seconds before restarting the infusion. If the rats did not drip the solution out within one minute, the infusion was continued and interrupted again for 30 seconds when the animal dripped out the solution. This procedure was repeated until the criterion, the rejection of the solution within one minute after a 30 second interruption of the infusion, was fulfilled. Most rats stopped ingesting the solution within the first minutes after having rejected it once.

**Preparation of experimental peritoneal dialysis solutions and buffer solutions**

All solutions tested in these studies were made in our laboratory and had the same concentrations of electrolytes (Table 1). Solutions tested included glucose-based PD solutions (containing 1.36%, 2.27% or 3.86% glucose, lactate 40 mmol/L, pH 5.5), mannitol-based PD solutions (containing 1.36%, 3.86% mannitol, lactate 40 mmol/L, pH 5.5) and lactate buffer solution (containing lactate 40 mmol/L, without glucose, pH 5.5). The solutions were either sterilized using a bacterial filter (Sterile 0.22 μm; Millipore S.A., Molsheim, France) or using heat (121°C, 45 min). In experiment 5, glucose-based PD

| **Table 1. Characteristics of different peritoneal dialysis solutions** |
|------------------|------------------|------------------|
| **Glucose based** | **Mannitol based** | **Buffer control** |
| Lactate mmol/L    | 40               | 40               | 40               |
| Sodium mmol/L     | 132              | 132              | 132              |
| Calcium mmol/L    | 1.25             | 1.25             | 1.25             |
| Magnesium mmol/L  | 0.25             | 0.25             | 0.25             |
| Chloride mmol/L   | 95               | 95               | 95               |
| pH                | 3.0, 5.5, or 7.4 | 5.5              | 5.5              |
| Osmotic agent %   | 1.36, 2.27 or 3.86 | 1.36 or 3.86    | 0                |
| Osmolarity mOsm/L | 346 or 396 or 484 | 348 or 486       | 246              |
| Sterilization     | Heat or filter   | Heat or filter   | Heat             |
solutions were sterilized with heat at pH 3.0, pH 5.5 and pH 7.4, and were then adjusted to neutral pH using sodium hydroxide before the IP infusion.

Experiments on ingestive behavior

The i.o. intake was measured 30 minutes after IP infusion of the test solutions. The i.o. infusion delivered the sucrose solution (1 mol/L) at the speed of 1 mL/min. For each experiment, the rats served as their own controls, using (except for experiment 3) the lactate buffered solution without glucose as the control solution.

Experiment 1: Effect of heat-sterilized glucose-based PD solutions. Eight rats (N = 8 for each solution) were given an IP infusion with 30 mL of glucose-based heat-sterilized PD solutions (1.36%, 2.27% or 3.86% glucose) and control solution (lactate buffered solution without glucose), respectively. The rats were tested in random order on four consecutive days.

Experiment 2: Comparison of heat-sterilized and filter-sterilized glucose-based PD solutions. Twenty-four rats were randomly divided into three groups (1.36%, 2.27% or 3.86%). The rats (N = 8 for each glucose concentration group) were given an IP infusion with 30 mL of filter sterilized or heat sterilized PD solutions, and tested in random order on two consecutive days.

Experiment 3: Comparison of filter-sterilized mannitol-based and glucose-based PD solutions. Sixteen rats were randomly divided into two groups (1.36% or 3.86%). The rats (N = 8 for each group) were treated with 30 mL 1.36% glucose or 1.36% mannitol based filter-sterilized solutions respectively, using the lactate buffered solution without glucose as the control, and were tested in random order on three consecutive days. This test was repeated using the 3.86% glucose or 3.86% mannitol-based filter solutions.

Experiment 4: Comparison of heat-sterilized mannitol- and glucose-based PD solutions. Sixteen rats were randomly divided into two groups (1.36% or 3.86%). The rats (N = 8 for each group) were given 30 mL 1.36% glucose-based or mannitol-based heat-sterilized solutions, respectively, using the lactate buffered solution without glucose as the control, and were tested in random order on three consecutive days. This test was repeated using 3.86% glucose-based or mannitol-based heat-sterilized solutions.

Experiment 5: Effect of pH during heat sterilization. Twenty-four rats were randomly divided into three groups (1.36%, 2.27% or 3.86%). The rats (N = 8 for each group) were given 30 mL 1.36% glucose based PD solutions, heat sterilized at pH 3.0, pH 5.5 and pH 7.4, respectively, using the filter-sterilized buffer solution as a control. The solutions were tested in random order on four consecutive days. This test was repeated in the same way using 2.27% and 3.86% glucose solutions.

Data analysis

The results are expressed as mean ± SD and analyzed using the paired t test for comparison between two groups and ANOVA for comparison between three or more groups with the aid of the Statview statistical program (SAS Institute, Inc., Cary, NC, USA) for a personal computer.

RESULTS

There was a significant decrease in i.o. intake following the IP infusion of glucose-based heat-sterilized PD solutions compared with the control buffer solution (Fig. 1). The i.o. intake was inversely related to the dialysate concentration of glucose (Fig. 1).

The i.o. intake was less reduced following the i.p. infusion of the filter-sterilized PD solution than after infusion with the heat-sterilized PD solutions for each of the different glucose (1.36%, 2.27% and 3.86%) PD solutions (Fig. 2).

There were no significant differences in i.o. intake between the IP infusion of glucose-based and mannitol-based filter-sterilized 1.36 and 3.86% solutions, although both the glucose-based and mannitol-based 3.86% solutions resulted in significantly lower i.o. intake than the buffer control solution (Fig. 3). However, there were significant differences in i.o. intake following the IP infusion of glucose-based and mannitol-based heat-sterilized PD solutions for both the 1.36% and 3.86% solutions (Fig. 4), and there were also significant differences in i.o. intake between infusion with 1.36% and 3.86% glucose-based and mannitol-based heat-sterilized solutions on the one hand, and, on the other hand, the buffer control solution. These results suggest indirectly that GDPs in-
Fig. 2. Effects of heat-sterilized (■) and filter-sterilized (□) PD solutions on intraoral intake of sucrose. The i.o. intake differed \((P < 0.05)\) between rats (mean ± SD, \(N = 8\)) receiving heat sterilized PD solutions and rats (\(N = 8\)) receiving heat sterilized PD 1.36%, 2.27% and 3.86% solutions glucose, respectively.

Fig. 3. Effects of control (■), glucose-based (□) and mannitol-based (□) filter-sterilized PD solutions on intraoral intake of sucrose. The i.o. intake in rats (mean ± SD, \(N = 8\)) was significantly reduced \((P < 0.05)\) following IP infusion of 3.86% glucose and 3.86% mannitol based solutions compared with the control solution (lactate buffer without glucose), but there were no significant differences between the 1.36% mannitol-based, 1.36% glucose-based PD solutions and the buffer solution. The i.o. intake did not differ between the mannitol based and glucose based 1.36% and 3.86% PD solutions, respectively.

The i.o. intake (mean ± SD) in rats (\(N = 8\)) in each group receiving glucose-based 1.36%, 2.27% and 3.86% PD solutions that were heat-sterilized at different pH values (control; ■ pH 3.0; (□) pH 5.5; (□) pH 7.4), using the filter-sterilized buffer solution as control, was influenced by pH during heat sterilization. There were significant differences among the different pH values (pH 3.0, pH 5.5, pH 7.4) in the 1.36%, 2.27% and 3.86% glucose-based solutions. The higher the pH value of the solution was during the heat sterilization, the lower the intraoral intake the solution induced.

DISCUSSION

Glucose is still the most commonly used osmotic agent in PD solutions although alternative osmotic agents (icodextrin and amino acids) have recently been introduced. Glucose is a main source of energy in the human body, and thus glucose in itself in physiologic concentration can hardly be considered toxic; however, the high concentration of glucose in PD solutions has several biologi-
cal side effects such as growth inhibition of cultured cells [21]. Furthermore, since glucose, especially d-glucose, is not a stable compound, it can undergo a variety of spontaneous degradation reactions in vivo, resulting in the formation of a variety of reaction products that are present in healthy subjects and in higher concentrations in uremic patients [22, 23]. Accelerated glucose breakdown also can take place in vitro, and during heat sterilization of the dialysate fluid, the formation of glucose degradation products (GDPs) is highly dependent on conditions such as temperature, duration of sterilization, glucose concentration and pH value [12, 23]. GDPs are toxic, and several investigations of the toxicity of GDPs in PD solutions have demonstrated an effect of GDPs on the function of cells in the peritoneum [22, 24].

Our previous study, using the same in vivo experimental appetite rat model as in the current study, found that a pH-neutral, bicarbonate/lactate buffered PD solution with a low concentration of GDPs resulted in less inhibition of appetite than a conventional low pH, lactate-buffered PD solution with high concentration of GDPs [17, 18]. We also showed that the lesser effect on appetite of the former solution was partially due to a lower concentration of lactate in this solution. However, the possible roles of glucose and GDPs have not previously been investigated.

In the present experiments, using the same experimental appetite rat model, we focused on the effects of glucose and glucose degradation on ingestive behavior. Firstly, glucose-based heat-sterilized PD solutions (made in our laboratory) were tested using different concentrations of glucose. The results show that heat-sterilized PD solutions suppress food intake, and that the degree of appetite inhibition is proportionate to the concentration of glucose. Thus, glucose itself or its breakdown products, or both, are involved in the process of inhibition of food intake during PD.

In order to investigate the possible separate role of GDPs in appetite inhibition, we then compared the effects of filter-sterilized PD solutions with heat-sterilized PD solutions on ingestive behavior. The results showed that the rats given heat-sterilized PD solutions consumed a smaller volume of i.o. sucrose than rats given filter sterilized solutions. Although we did not measure the GDPs of the dialysate, it seems likely that degradation of glucose during heat sterilization of PD solutions contributed to the suppression of ingestive behavior.

It is conceivable that this appetite suppression is related to the rapid uptake of glucose, GDPs and lactate in the portal circulation, resulting in high concentrations of absorbed substances in the splanchnic area, and subsequent stimulation of hepatic glucoreceptors and secretion of cholecystokinin-8, glucagon-like peptide 1, insulin and other mediators of appetite suppression [25–27].

Heat sterilization has to be performed at a low pH to reduce the caramelization of glucose, which otherwise would turn the fluid brownish, and in standard PD fluids the pH is therefore usually set at 5.0 to 5.5 before sterilization. Lower pH during heat sterilization results in less formation of GDPs. For example, when the d-glucose concentration was 3.86%, 324 μmol/L 3-deoxyglucosone (3-DG) was formed under standard sterilization conditions (pH 5.5). On the other hand, when the 3.86% glucose PD fluid was heat sterilized at pH 3, which can be achieved in a two-chamber bag, 3-DG concentration was reduced to 42 μmol/L [22].

We therefore prepared glucose based PD solutions that were heat sterilized at pH 3.0, 5.5 and 7.4. The pH value of these solutions was corrected to neutral pH before instillation intraperitoneally to avoid any possible effect of pH as such on appetite. The results showed that the amount of i.o. intake was inversely related to the pH value of PD solutions during heat sterilization; the higher the pH of the solution during heat sterilization, the stronger the appetite inhibition. This suggests a strong impact of GDPs on appetite.

Mannitol and glucose have almost the same molecular weight, but mannitol is more stable during heat sterilization [28, 29], and therefore was utilized (as an isoosmolar control) to compare the effect of isoosmolar glucose-based and mannitol-based PD solutions on appetite. Our results demonstrated that there was no significant difference between glucose-based and mannitol-based PD solutions using filter sterilization, although both glucose-based and mannitol-based filter sterilized solutions suppressed appetite compared with the buffer control solution. Thus, the osmolality of the PD solution may influence appetite, and this effect appears to be similar with glucose and mannitol. In contrast, a significant difference was found between glucose-based and mannitol-based PD solutions if both were heat sterilized, suggesting an impact of GDPs. In addition, we noted that the mannitol solution was colorless while the glucose solution had a brown color after heat sterilization. This indicates indirectly that glucose breakdown products formed during heat sterilization contributed to hypophagia. On the other hand, glucose-based and mannitol-based filter-sterilized PD solutions inhibited appetite to the same degree compared with the control buffer solution, suggesting that hyperosmolality also was involved in suppression of appetite during the short intraperitoneal dwell.

Lower pH, higher glucose concentration and the removal of catalyzing substances such as calcium and magnesium during sterilization are known to minimize the cytotoxic glucose breakdown [12, 14]. In the two-compartment bag or three-chamber bag that are now available for PD fluids, the high concentration of glucose in one compartment can be separated from the catalyzing substances such as calcium and magnesium during steril-
ization, and this can minimize generation of GDPs [14, 30]. The biocompatibility of these fluids previously was reported to be improved with regards to basal cytotoxicity, inflammatory response, and concentration of GDPs. In the clinic, the use of the new PD fluids produced in a two-compartment bag has been shown to reduce pain during infusion and longitudinal studies have shown signs of improved peritoneal viability such as an increase in the dialysate concentration of cancer antigen 125, a marker of mesothelial cell mass [31, 32]. Whereas most previous studies were focused on the local effects of PD fluids on the peritoneal cells, the present studies suggest that the bioincompatibility of heat-sterilized PD solutions also might have a systemic impact on food intake and thus on nutrition. However, the possible clinical relevance of this finding has not been studied to date. In summary, glucose-based heat-sterilized PD solutions apparently inhibit ingestive behavior more than the glucose-based filter-sterilized PD solution, the suppression of intake being proportional to the concentration of dialysate glucose and inversely related to the pH value in the PD solution during heat sterilization. This suggests that appetite in PD patients might be improved if the dialysate concentration of GDPs were reduced by modifying the method of sterilization, by lowering the pH value during heat sterilization, and by separating the different compounds during sterilization. New PD solutions with low concentrations of GDPs (and with low lactate and neutral pH) may be beneficial with regards to reduced appetite inhibition, and therefore may contribute to an improved nutritional status of patients undergoing peritoneal dialysis.

ACKNOWLEDGMENTS

This study was supported by a grant from Baxter Healthcare (McGaw Park, IL, USA). We thank Mrs. Monica Eriksson and Mrs. Ann-Christin Bragfors-Helin for the technical help in the preparation of PD solutions. We appreciate the support from the Clinical Research Center.

Reprint requests to Dr. Bengt Lindholm, Divisions of Baxter Novum and Renal Medicine, K-56, Huddinge University Hospital, S-14186 Huddinge, Stockholm, Sweden. E-mail: bengt.lindholm@klinetki.se

REFERENCES

28. PHANICHPHANT S, GOUTHAPRAPONG P: Short-term effect of 4% hyper-

