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Research Article

Reducing the formation of glucose degradation products in peritoneal dialysis solutions by ultrahigh temperature ohmic heating

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Peritoneal dialysis (PD) is commonly performed by using preprepared dialysis solutions containing glucose, which are thermally treated to achieve commercial sterilization. A series of glucose degradation products (GDPs) are being formed, which react with the tissue during the dialysis procedure, thus baring a negative effect on the patient and the dialysis process. The present study tested the efficacy of ohmic heating as an alternative thermal treatment for continuous sterilization of PD solutions. The process was compared to conventional retort treatment, and GDPs accumulation was measured. Thermal treatments using the ohmic heating system were performed at three temperatures (105, 125, and 150°C) with residence time at each temperature ranging from 0.84 to 12.0 s. The resulting concentrations of glyoxal (GO), methylglyoxal (MGO), and 3-deoxyglucosone (3-DG) in the PD solutions were measured. None of these GDPs were found in PD fluids treated by ohmic heating at 105°C. The concentration of 3-DG, after a standard sterilization treatment (121°C, 20 or 40 min) was one order of magnitude higher (~140 and 242 μM) than after ohmic heating treatment at 125°C. The results of the present study suggest that this technique can be used to produce solutions with much lower content of GDPs. It also demonstrates the advantage of using the ohmic heating technology as a tool for high temperature short time treatment of PD fluids.

Keywords: Glucose degradation / Ohmic heating / Peritoneal dialysis

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1 Introduction

Peritoneal dialysis (PD) has developed into an effective renal replacement therapy for treating patients with kidney failure [1]. The PD fluids contain an osmotic agent, glucose, which is most widely used because it is cheap and nontoxic. Glucose is easily metabolized and is the most important energy source for living cells. Before use, PD fluids are sterilized, normally by exposure of the fluids to high temperatures (approximately 120°C) for a significant time (20–40 min) [2]. The sterilization at these temperatures can generate reactive intermediates that induce glycation in the patients on PD. It involves a complex series of parallel and sequential reactions, often termed the "Maillard reaction" [3]. This reaction can undergo a cascade of slow reactions to form irreversible glycation endproducts (AGEs). Much attention has recently been paid to these advanced glycosylation endproducts relating to aging or chronic complications of diabetes [4–6].

During the conventional sterilization process, glucose degrades to carbonyl compounds, forming intermediate active substances, which are generally referred to as glucose degradation products (GDPs). Several GDPs have been identified including glyoxal (GO), methylglyoxal (MGO), 3-deoxyglucosone (3-DG), 3,4-dideoxyglucosone-3-ene (3,4-DGE), formaldehyde, and 5-hydroxymethyl furaldehyde (5-HMF) (Fig. 1). However, the number of identified GDPs represents only a fraction of a large number of compounds that can be generated from glucose. 3,4-DGE was recently identified as the most biologically reactive GDP in PD fluids [2].

The GDPs have been reported as cytotoxic in a great variety of *in vitro* and *in vivo* models [1, 7–10]. GDPs have been shown to cause severe inhibition of cell growth of L-929 fibroblasts [8] and mesothelial cells [8, 11–14]. It was also

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Abbreviations: 3-DG, 3-deoxyglucosone; **3,4-DGE,** 3,4-dideoxyglucosone-3-ene; **GDPs,** glucose degradation products; **GO,** glyoxal; **MGO,** methylglyoxal; **PD,** peritoneal dialysis

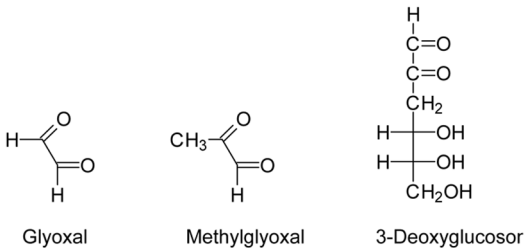

Figure 1. Chemical structure of GDPs: glyoxal, methylglyoxal, and 3-DG.

demonstrated that several glucose-derived aldehydes were capable of directly inhibiting cell growth in culture [15]. Most GDP studies to date showed cytotoxicity with animal and/or transformed cells including mouse L929 fibroblasts [8, 16–18], RAW 264.7 macrophages [19], and human neuroblastoma SH-SY5Y cell line [19].

An alternative method for sterilizing the PD fluids is the ohmic heating process, which involves the passage of alternating current though the fluid, thus generating internal heat as a result of electrical resistance [20]. This technology provides rapid and uniform heating, resulting in less thermal damage to the product [21]. In addition, the absence of a hot surface in ohmic heating reduces fouling problems and thus also thermal damage to the fluid being sterilized. A high-quality product with minimal structural, nutritional, or sensory changes can be manufactured in a short operating time [22]. One key to the successful implementation of an ohmic process is the rate of heat generation, the electrical conductivity of fluid material, and the way the fluid flows through the heater. A small number of studies conducted on ohmic heating were done on food materials such as vegetable samples, meat, tomato, and orange juice [23]. In these studies it was shown that electrical conductivities of tomato and orange juice increase linearly with temperature and decrease with solids content.

Lima *et al.* [24] studied the degradation of vitamin C in orange juice subjected to electrical and conventional heating. Experiments were preformed using a static ohmic heating apparatus, and matching heating histories were applied for both conventional and ohmic heating. A statistical analysis showed that electrical field had no significant effect on ascorbic acid degradation. A continuous alternating current electric field was applied to orange juice containing *Bacillus subtilis* spores in order to examine the inactivation effect of electrical equipment [25]. Electrical treatment at 121°C under pressurized conditions was found to reduce the viable *B. subtilis* spores in orange juice. In two studies conducted by Leizerson and Shimoni [26, 27], the effects of ohmic heating on the quality of orange juice were examined and compared to those of heat pasteurization at 90°C for 50 s. Microbial counts showed complete inactivation of bacteria, yeast, and mold during ohmic and conventional treatments. Ohmic-heated orange juice maintained higher amounts of the five representative flavor compounds than in heat-pasteurized juice. Sensory evaluation tests showed no difference between fresh and ohmic-heated orange juice. It was also shown that although both thermal treatments prevented the growth of micro-organisms for 105 days, the sensory shelf life of ohmic-heated orange juice was >100 days and was almost two times longer than that of conventionally pasteurized juice.

The objective of our study was to investigate the effects of ohmic heating on the degradation of glucose in PD fluids and to monitor the concentration of GDPs mainly: GO, MGO, and 3-DG, and to compare with PD fluids that had been subjected to the standard autoclave sterilizing heat treatments.

2 Materials and methods

2.1 Composition of PD solution

Laboratory-made PD fluids were produced and the pH was adjusted to 6.6. The fluid contained: glucose 4.00%, NaCl 0.58%, sodium lactate 0.50%, $CaCl_2$ 0.03%, and $MgCl_2$ 0.005%. The PD fluid reagents were purchased from Sigma–Aldrich Chemical (Rehovot, Israel).

Standards of GO, MGO, and 3-DG were purchased from Sigma–Aldrich Chemical. All other reagents were of analytical grade.

2.2 Conventional sterilization

PD fluids samples (200 mL) were filled into glass jars, capped, and then heat-sterilized by autoclaving for 20 min at 120°C and 40 min at 120°C.

2.3 Thermal treatment by ohmic heating

The PD fluids were thermally processed in a 50 kW pilot scale Electro-heating™ system (Raztek, Sunnyvale, CA; Fig. 2). The system consists of two feeding tanks: one for a salt solution and the other for the untreated product. The untreated product continuously enters the system *via* a mono pump (A.P.V. Baker, Peterborouth, UK). Initially, the product is pumped to the first part of the system, the rapid cooler where the product is preheated [28, 29]. The rapid cooler consists of a tank containing two sets of coiled tubes: an upper coil tube for the untreated product flow, and a lower coil for the heated product flow. The upper part of the tank is saturated with steam, and the lower part is filled with water, and the whole system is under vacuum to maximize the heat transfer. The hot product passes through the tube, the water is quickly boiled, and the cold product passes through the tube above the boiled liquid, and is heated by the steam condensation. Following preheating, the product enters the electroheater at a temperature which

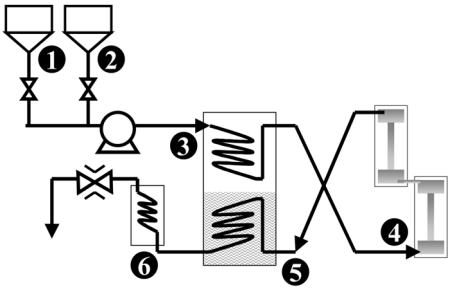

Figure 2. Scheme of the electroheating system, including: salt solution tank (1), product tank (2), preheating (3), electroheating (4), rapid cooling (5), and secondary cooling (6).

is the exact mean between its temperature at the tank (point 3, Fig. 2) and the treatment temperature (point 5, Fig. 2) [30–33]. The electroheating unit consists of two pairs of adjacent graphite electrodes, with a 20-cm gap between each pair of electrodes. The product flows along the axis between the electrodes. The system utilizes alternating current at a frequency of 50 Hz, and at maximum voltage of 8 kV. The system controller automatically determines the necessary current and voltage using a feedback from a thermocouple at the exit from the heating chamber to heat the product to the predetermined treatment temperature. Following electroheating, the product enters a 120 cm holding tube, in which the thermal treatment takes place. This tube connects the exit of the electroheater to the entry of the rapid cooler (point 5, Fig. 2). Then the product is cooled down rapidly in the lower part of the rapid cooler. Finally, the product is cooled down to room temperature in a tubular heat exchanger.

The installation was pressurized with a pressure valve to provide a backpressure of ~12 atm, and prevent boiling of the superheated product. A presterilization step of the electroheating system was carried out by circulating a sodium chloride solution with the same electrical conductivity of PD fluid (σ = 0.36 S/m at 25°C) at 120°C for over 20 min. PD fluids were treated by the ohmic heating system at set point temperatures of 105, 125, and 150°C, and in various treatment times. The *F*-values were calculated with 120°C as the reference temperature, on the basis of z = 10°C.

2.4 GDPs analysis

Analyses of MGO, 3-DG, and GO concentration in PD fluids was performed according to the method of Okabe *et al.* [34]. Samples were eluted with gradient elution as quinoxaline after derivatization with 2,3-diaminonaphthalene. The test solution (0.5 mL) was mixed with 0.5 mL of 0.1 mol/L sodium phosphate buffer (pH 7.2) and 0.1 mL of 0.1% diaminonaphthalene solution in methanol. The reaction mixture was incubated overnight in the dark at room temperature. The analysis was conducted using an RP-HPLC, HP 1100, equipped with a diode-array detector at 268 nm, controlled by ChemStation software package (Hewlett-Packard, Wilmington, DE). The elution was done using a discontinuous gradient (ACN–water). The flow rate was set at 1 mL/min and the ACN concentration was adjusted to 25% during the first 14 min, to 65% for the next 16 min, and back to 25% for the final 5 min. The quantification was done using calibration curves, with detection limit of <0.1 μM for all GDPs.

3 Results and discussion

The main objective of the present study was to evaluate the effect of thermal treatments by continuous ohmic heating on the formation of GDPs in PD fluids. Initially, the PD fluids were treated by conventional sterilization. The concentration in PD fluids post a standard sterilization treatment (121°C, 20 and 40 min) was 140 and 242 μM for of 3-DG, and 11 and 36 μM for MGO, respectively. In addition, GO was not detected when sterilized in the conventional treatments. These results are in line with previously reported concentrations, by Erixon *et al.* [2] and Okabe *et al.* [34]. This observation indicates that the composition being used in this study as well as the analytical procedures is adequate.

Thermal treatments using the ohmic heating system were performed at three temperatures (105, 125, and 150°C) with residence time at each temperature ranging from 0.84 to 12.0 s. The resulting concentrations of GO, MGO, and 3-DG in the PD solutions were measured by HPLC. None of these GDPs was found in PD fluids treated by ohmic heating at 105°C. The results for fluids treated at 125 and 150°C are presented in Figs. 3A and B, respectively.

It can be seen that concentration increases with the time of exposure to the heat treatment. The concentration of 3-DG, after a standard sterilization treatment (121°C, 20 and 40 min) was one order of magnitude higher (~140 and 242 μM) than after 12 s of the ohmic heating treatment at 125°C (Fig. 3A). In addition, GO was not detected when sterilized in the conventional treatments and showed minor concentration after the ohmic heating treatment. MGO concentrations were 11 and 36 μM after 20 and 40 min treatment, respectively, but were not detected after ohmic heating treatments at 125°C. As could be expected, at 150°C, higher concentrations of all three GDPs are accumulated (Fig. 3B). For 3-DG and MGO, a fast accumulation is observed up to final concentrations of approximately 130 and 3 μM, respectively. An interesting result was obtained for GO, where a sharp increase in its concentration was detected during short thermal treatments (up to *ca.* 8 μM), falling to *ca.* 3 μM with treatment time exceeding 2 s. This result may be attributed to its nature as an intermediate in the degradation process of glucose.

These observations, however, do not provide the most accurate and useful data on the ohmic heating process, as

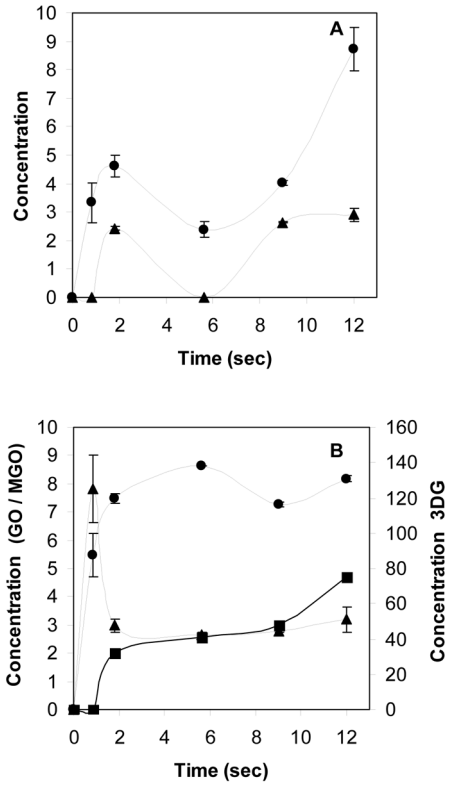

Figure 3. Formation of 3-DG (●), GO (▲), and MGO (■) in PD fluids during ohmic heating treatment at (A) 125 and (B) 150°C (μM) ($n = 3$).

compared to conventional commercial sterilization. The extent of thermal treatment is usually defined as a function of time and temperature. The common term being the equivalent time at a reference temperature. For this reason, we have calculated the equivalent time at 120°C for all the ohmic heating treatments, in order to compare them to the conventional treatments reported in the literature, using the common function

$$F^z_{T_1} = F^z_{T_2} \times 10^{\frac{T_2 - T_1}{z}}$$

where z is the temperature increase needed to accomplish a 1-log cycle reduction in the time required for a one-log cycle reduction in the microbial population, T_1 is the reference temperature (120°C in this study), and T_2 is the treatment temperature, $F^z_{T_1}-$ is the total time required to obtain similar thermal killing effect under specified z at the reference temperature T_1, $F^z_{T_2}-$ is the treatment time at T_2. To facilitate the comparison of the ohmic heating with conventional sterilization performed in this study or reported in the literature we present all the data in Figs. 4A–C.

It can be seen from Fig. 4 that the ohmic heating system reduces the final concentration of GDPs in PD solutions as compared to a standard sterilizing process.

When the three different ohmic heating treatments are compared, an increase in F-value during the treatment at

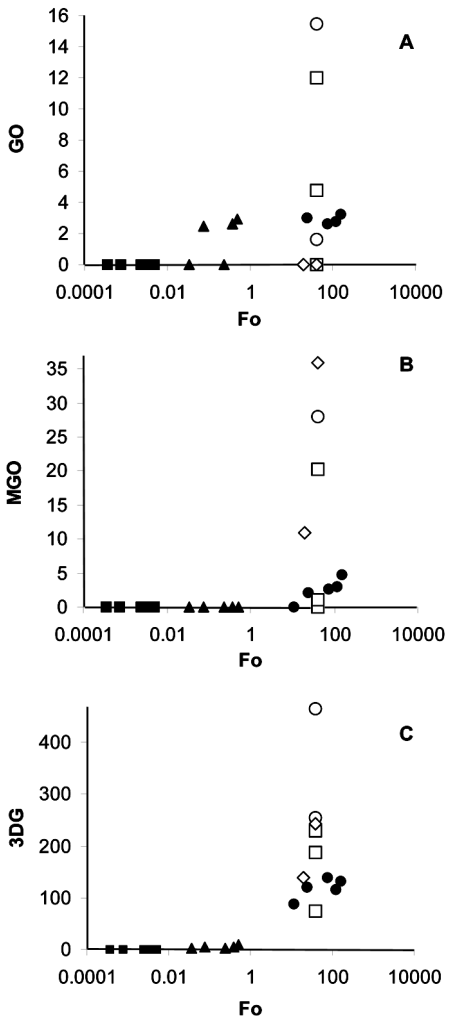

Figure 4. Comparison of different thermal treatments on (A) GO, (B) MGO, and (C) 3-DG concentration (μM). Thermal treatments are expressed as F-value (min). Temperatures of the ohmic heat treatments: 105° (■), 125° (▲), and 150°C (●). Heat sterilizing in stream autoclave at 121°C in the present study (◇), and in previous studies with glucose composition of (○) ~4 and (□) ~1.5% glucose [34].

150°C showed an increase of ~43% in the concentration of 3-DG. The other GDPs studied, MGO and GO, showed a minor but significant increase in their concentration at all temperatures (105, 125, and 150°C) of the ohmic heat treatments at high F-values (>100). It appears that using the ohmic heating at 125°C does not form at all or form minor concentrations of the GDPs tested. When these results are compared to a study conducted by Erixon *et al.* [2] it was observed that immediately after sterilization at 121°C for 40 min ($F_0 = 40$), the concentration of 3-DG was ~230 μM. In the present study, the maximal concentrations were 0, ~9, and ~131 μM at temperatures of 105, 125, and 150°C respectively, after 12 s of ohmic heating treatments. In addition, the concentration of GO in that study was three-fold

the concentration in the ohmic heating treatment. MGO concentration was comparable in both studies. Okabe *et al.* [34] studied the effects of sterilization of PDF samples by filtration and heat sterilization (121°C, 40 min, $F_0 = 40$). The concentration of 3-DG in PDF sample, adjusted to pH 5.5 and 6.7 with glucose concentration of ~4% after sterilization, was 465 and 254 μM, respectively. The heat sterilization processes in the present work (121°C, 20 and 40 min) result in 140 and 242 μM for of 3-DG, and 11 and 36 μM for MGO, respectively. When the ohmic heating treatment was applied in the present study, the concentration at similar *F*-values was minor. The concentrations of MGO and GO in the study of Okabe *et al.* [34] were of five-fold the concentration after the ohmic heating treatments (Fig. 4). In addition, Linden *et al.* [35], reported that a considerably active GDP, 3,4-DGE, was present in conventionally manufactured PD fluids at a concentration of ~22 μM. These results are two-fold higher than the concentrations of 3-DG after the ohmic heating treatment. It should be noted that we have quantified only three dicarbonyls, and although we did not detect other quinoxaline peaks during the analysis, it is still possible that the ultrahigh temperature heating can selectively induce other types of dicarbonyls not being targeted in this study.

The results obtained in the present study clearly demonstrate the advantage of using the ohmic heating technology as a tool for high-temperature short-time treatment of PD fluids that will reduce the formation of toxic GDPs. An important question is, however, whether the beneficial effect is due to the heating by the ohmic heating system, or merely the fact that the fluid was treated by ultrahigh temperature-short time treatment (UHTST). While ohmic heating has proved itself as an efficient means to reduce thermal abuse of sensitive compounds in solution, one should keep in mind that it is the difference in activation energy between thermal inactivation of pathogenic bacteria and the activation energies of GDPs formation in PD fluids. If this is the case, then such sensitive solutions can be treated efficiently by any UHTST system. For this purpose, however, much more data of the kinetics and temperature sensitivity (*e.g.* activation energy) of GDPs formation is required.

4 Concluding remarks

The results of the present study suggest that thermal treatment by continuous ohmic heating can be used to effectively sterilize PD fluids while forming minor concentrations of GDPs as compared to the standard sterilization process. The reduction in GDPs formation could be as a result of lower thermal abuse by overcoming heat transfer problem that are common when using conventional heat exchangers. It may also be, however, the consequence of lower temperature dependence (Ea) of GDP formation compared to the thermal sensitivity of microbial inactivation rate. This point can only be clarified if the kinetic parameters of GDPs formation and their temperature dependence will be available. Further studies are being conducted using the ohmic heating system to provide this type of data.

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