Advanced Glycosylation End Products in Diabetic Rats on Peritoneal Dialysis with Different Solutions

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INTRODUCTION

Continuous ambulatory peritoneal dialysis (CAPD) has been used to treat patients with end-stage renal failure for about 20 years now. Glucose has been the osmotic agent in all peritoneal dialysis solutions. Problems associated with the use of glucose include : A short duration of effective ultrafiltration, particularly in high transporters[1], the potential for disruption of the peritoneal membrane[2], impairment of peritoneal cellular defense mechanisms[3], and adverse metabolic and nutritional consequences of the daily absorption of 150-300 grams glucose[4]. Dobbie suggested the need for a CAPD solution which is iso-osmolar with uremic serum and devoid of the damaging effects associated with glucose solutions[5]. Icodextrin has been developed in response to these requirements.

Icodextrin is a starch-derived glucose polymer with an average molecular weight of 16,200 daltons and with chain lengths varying between 4 and 250 glucose units. Icodextrin, a 7.5% icodextrin based dialysis solution has an osmolality of 284 mOsm/kg and the pH is 5.3[6]. Potentially, the iso-osmolar Icodextrin solution should be less damaging to the peritoneum and to the local host defenses as compared to the hyperosmolar glucose solutions. Icodextrin has a "colloid"-induced osmotic convective transport as compared to the "crystalloid osmosis" of glucose-based solutions. The Icodextrin solution provides sustained ultrafiltatation over long dwell times of 8-12 hours in CAPD[7] and up to 16 hours in automated peritoneal dialysis[8]. Due to these properties, Icodextrin can be considered a suitable alternative for glucose-based solutions in the treatment of patients with ultrafiltration failure[9].

Glycosylation refers to a non-enzymatic process in which reducing sugars covalently bond to molecules, which contain free amino groups, such as peptides and proteins. *In vivo* the prominent sugar involved is glucose and the rate of glycosylation is dependent primarily upon the glucose concentration. The carbonyl group of an open-chain glucose molecule reacts with an amino group to form an unstable, reversible Schiff base intermediate. The Schiff base may then undergo an Amadori rearrangement to form a stable ketoamine (Amadori product). This reaction reaches equilibrium in approximately 28 days[10]. Over a period of months, the Amadori product will subsequently undergo further chemical reactions involving oxidation, dehydration, cyclization, rearrangement and condensation with other reactive amino groups to form advanced glycosylation end products (AGEs) which irreversibly bind to protein[11].

AGEs are a heterogenous group of compou-

nds characterized by : 1) the ability to polymerize proteins; 2) a fluorescent spectrum; and 3) a yellow-brown color, hence, the term browning reaction. Once the initial glycosylation reaction has occurred, polymerization and cross-linking continue even in the absence of glucose[12]. The main sites of AGEs cross-linking are the long-lived structural proteins, such as those found in the tissue matrix and basement membranes. Tissue concentration of AGEs increase naturally with aging and has been shown to be accelerated in patients with diabetes mellitus as a consequence of hyperglycemia. Elevated serum concentrations of deoxyglucosones, which are reactive intermediates in AGEs formation, and have been detected in diabetics[13, 14] and patients on dialysis[15].

AGEs have been implicated in the pathogenesis of some of the secondary complications of diabetes[16], especially vascular disease. Effects of AGEs may also be mediated through interaction with receptors on endothelial, smooth muscle, mesangial and inflammatory cells, among others[17]. Increased serum AGEs have been shown to contribute to the accelerated vascular dysfunction associated with uremia and diabetes[18, 19].

It has been proposed that the formation of AGEs in diabetes results in cross-linking of collagen and distortion of subcelluar structures, resulting in irreversible tissue damage of the peripheral nerve, and macro- and microvasculararture[20, 21]. Prolonged exposure to glucose-based dialysis fluids currently used results in production and deposition of AGEs in the sub-endothelial area of the peritoneum, microvascular changes like those seen in diabetics, and finally result in the loss of peritoneal function. Several *in vivo* and *in vitro* studies have demonstrated that glucose-based solutions result in higher AGEs as compared to icodextrin-based solutions. *In vivo* studies on the formation of AGEs are limited to retina, nerve, aorta and glomerulus[22, 23]. Long-term peritoneal dialysis comparing icodextrin versus glucose and the formation of AGEs in an animal model have not been done. This study was performed to compare *in vivo* in the rat model of peritoneal dialysis, the effects of glucose-based solutions to those of icodextrin-based solutions with respect to : AGEs formation in the peritoneal membrane and the effects upon peritoneal transport characteristics.

MATERIALS AND METHODS

1. Animals

The study was performed on 42 male Sprague-Dawley rats weighing between 275 and 300 grams randomly divided into 5 groups : group C (n=6), control with catheter but no dialysis; group D (n=6), diabetic rats with catheter but no dialysis; group G (n=10), diabetic rats dialyzed with standard 2.5% glucose solution (Dianeal, Baxter Healthcare, Deerfield, IL, U.S.A.) for daytime exchanges (8 : 00 A.M., 2 : 00 P.M.) and 4.25% glucose solution for the overnight exchange (10 : 00 P.M.); group H (n=10), diabetic rats dialyzed with standard 2.5% glucose solution during daytime exchanges and 7.5% Icodextrin solution (Icodextrin, Baxter Healthcare, Castlebar, Ireland) for the overnight exchange; group I (n=10), diabetic rats dialyzed with 7.5% Icodextrin solution for all exchanges. One animal in group D, three animals in groups G and I, and two animals in group H were sacrificed before completion of the study due to non-functional catheters, leaving 33 animals for analysis.

2. Induction of Diabetes

Diabetes was induced using Streptozotocin (65 mg/kg; Sigma, St. Louis, MO., USA) dissolved in citrate buffer (0.1 M, pH 4.5) was

injected into the tail vein under methoxyfluorene anesthesia (Metofane, Pitman-Moore Inc., NJ, USA) in all groups except control group C. Diabetic rats with serum glucose greater than 500 mg/dl were given regular insulin (0.5-2 units) subcutaneously twice daily to maintain the serum glucose level between 250-500 mg/dl.

3. Catheter Implantation

The animal was placed in a large jar and anesthetized with methoxyfluorene and then anesthesia was maintained by a nose cone. The abdomen and the back of the neck were shaved and disinfected with Amukin (Amuchina, Genova, Italy). The area was then covered with Tegaderm (3-M cooperation, MS, USA). An incision was made in the skin along the midline 1cm below the xiphoid process and extending vertically approximately 2 cm. The peritoneal cavity was penetrated by blunt dissection 0.5 cm below the xiphoid. The tip of the catheter was then advanced into the cavity and secured with a single stitch through the cuff material and the superficial muscle layer. The procedure was performed carefully to avoid bleeding and trauma to the peritoneum. The opening into the peritoneal cavity was just large enough to allow the catheter to be pushed through such that the catheter fit snugly. With the use of a 3 mm trocar the other end of the catheter was tunneled subcutaneously to a point between the animal's scapulae. The trocar was then pushed through the skin, and the catheter pulled through the tunnel. The second cuff was located approximately 0.5-1.0 cm from the exit site. The catheter was trimmed leaving 1.5-2.0 cm exposed. The catheter was then capped. The animals were allowed to wake up, were placed in their cages, and allowed free access to food and water. Rats were given a single dose of bupremorphine subcutaneously for analgesia with an additional doses as needed. This therapy was continued for a period of 72 hours after surgery if necessary.

When a catheter malfunctioned due to omental wrapping, the catheter was left in place and a second catheter was implanted contralaterally with the same technique.

4. Dialysis Exchange

Dialysis was started on the first day with the instillation volume gradually increased from 15 to 25 ml. Prophylactic antibiotics (vancomycin 125 mg/L and gentamicin 32 mg/L) were given intraperitoneally in the solution until the fifth day postoperatively. The prophylactic antibiotics were changed every 5 days thereafter according to the sequence : Gentamicin 8 mg/L; vancomycin 25 mg/L; ciprofloxacin 25 mg/L; ceftazidime 125 mg/L; sulfamethoxazole/trimethoprim 200/40 mg/L. Dialysis exchanges were performed three times a day at 8 : 00 A.M., 2 : 00 P.M., and 10 : 00 P.M., seven days a week for 3 months.

5. Peritonitis

Samples for culture were collected once weekly, before the instillation of the morning dialysis solution. Diagnosis of peritonitis was based only on the culture results (more than 10 colonies in a blood agar plate) since, unlike in humans, there is no accepted definition of peritonitis. The infection was treated according to the sensitivity test result.

6. Peritoneal Membrane Function and Blood Tests

The peritoneal membrane function was assessed on the eighth day and at 1 month, 2 months, and 3 months by performing a one-hour peritoneal equilibration test (PET) using 1.5% glucose peritoneal dialysis solutions. 1.5%

glucose solution was used for the overnight exchange before the PET.

Installation of 25 ml of dialysis solution was done at time zero. At one hour the animals were anesthetized and a sample of dialysate was taken for urea nitrogen, creatinine, glucose. Immediately thereafter a blood sample (2 ml) was taken by direct cardiac puncture for urea nitrogen, creatinine, glucose, lipid profile, and hematocrit. In case of peritonitis the test was deferred until the cultures were negative.

Peritoneal membrane transport rate was assessed by dialysate to plasma ratio (D/P) of urea nitrogen and D/Do of glucose, where D is the glucose concentration in dialysate after a one hour dwell and Do is glucose concentration in dialysis solution before infusion into the peritoneal cavity. High D/P ratios of urea and low D/Do ratios of glucose indicate high transport and vice versa.

7. Weight

All animals were weighed at the induction of diabetes, on the eighth day, and at 1, 2, and 3 months, before the morning dialysis exchange.

8. Histologic Analysis

Specimens for histologic examination were obtained from all animals at the time of sacrifice during the 12^{th} week. Peritoneal tissues were obtained from the following four locations : A loop of gut with mesentery, abdominal wall, diaphragm, and surface of liver. The peritoneal membrane was evaluated by gross inspection and light microscopy. Gomori's trichrome stain was used to detect collagen deposition (fibrosis) in the peritoneal membrane. This procedure differentially stained connective tissue elements as follows, collagen-green, muscle tissue-red, and nuclei-blue to black.

9. Immunohistochemical Anlysis

At the end of the study animals were anesthetized and perfused transcardially with phosphate buffered saline (PBS) followed by 4% paraformaldehyde. Isolated tissues were put in 30% sucrose solution. The tissues were then rapidly frozen with isopentane (2, 3-dimethyl bytane) in liquid nitrogen, frozen tissue sections were cut sequentially at a 10 μm thickness and thaw-mounted onto slides.

Immunostaining of AGEs in peritoneal tissue sections was conducted using the streptoavidin-biotinylated peroxydase complex method. The sections were washed three times with PBS. Endogenous peroxydase activity was blocked by incubating the sections with 0.3% H_2O_2 at room temperature for 30 minutes. The sections were washed with PBS and incubated with normal goat serum in PBS at room temperature for 2 hours. After removing the normal goat serum, the sections were incubated with anti-AGE mouse monoclonal antibody (diluted×100 in PBS; Dojindo Laboratories, Tokyo, Japan) in a humid chamber at room temperature overnight. The sections were washed with PBS and incubated with secondary antibody, biotinylated anti-mouse IgG (Vector Laboratories, CA, U.S.A.) at room temperature for 1 hour. After washing three times with PBS, the sections were incubated with streptoavidin peroxydase complex (Vector Laboratories, CA, U.S.A.) at room temperature for 30 minutes. After washing three times with PBS, the reaction was completed by the addition of diaminobenzidine for 15 minutes. After washing with PBS, the slides were counterstained with hematoxylin. Control staining without primary antibody was done (2 sections in each group) to ascertain that no antibody was present in the tissues.

The slides were read by 5 different exami-

ners in a blinded fashion and staining intensity was graded semi-quantitatively from 0 to 3, with 0 representing no staining, 1 for weak, 2 for moderate and 3 for strong staining.

10. Statistical Analysis

Data are presented as mean±SEM. Statistical analysis of values at different time points among groups were assessed by one-way analysis of variance (ANOVA), with Tukey's correction for multiple comparisons using SigmaStat 2.0 (SPSS Inc.) for Windows. Differences were considered significant at a $p<0.05$.

RESULTS

1. Immunohistochmical detection of AGEs in the peritoneal membrane

The results of immunohistochemical staining showed that AGE staining was very minimal or absent in group C (Fig. 1A). In group D, the intensity of staining of mesothelial layers ranged from very weak to moderate. On the other hand, the staining of the vascular wall was weak to moderately positive in all cases. In interstitial and connective tissues, AGEs were not found. In group G, the mesothelial layers showed a consistent staining pattern ranging from moderate to strong. The staining of the vascular wall was moderately to strongly positive in all cases. No AGEs were found in interstitial or connective tissues (Fig. 1D). In group H, the mesothelial layers were stained in varying degree of intensity ranging from weak to moderate. The staining of the vascular wall was moderately to strongly positive in all cases. In interstitial and connective tissues, AGEs were not found (Fig. 1C). In group I, the mesothelial layers showed inconsistent staining patterns ranging from weak to moderate. The staining of the vascular wall was also weakly to moderately positive in all cases. AGEs were not found in interstitial and connective tissues (Fig. 1B).

The scores of AGEs staining in respective groups are shown in Fig. 2 The grade of immunostaining was lowest in group C and highest in group G. There were significant differences between group C (0.08±0.08) and groups G (2.27±0.28), H (1.72±0.19), and I (1.26±0.21), $p<0.001$, $p<0.001$ and $p=0.008$, respectively as well as between group G (2.27±0.28) and groups D (1.12±0.29) and I (1.26±0.21) $p=0.016$ and $p<0.05$, respectively and as well as between group C and D ($p<0.05$). A strong correlation was noted between the serum glucose concentration and the scores of AGEs stain ($r=0.894$)(Fig. 2A).

2. PET

Changes in D/Do glucose over time are shown in Table 1. A tendency to decreased D/Do glucose over time was noted. Group H had higher ultrafiltration compared to other groups. There were significant differences in D/Do between groups C (0.40±0.01) and H (0.35±0.01) $p<0.05$. Group H had higher ultrafiltration than groups G and I, but there was no statistical significance.

Changes in D/P urea over time are shown in Table 2. A tendency to increased D/P urea over time was noted. Group H had higher ultrafiltration compared to other groups. There were significant differences between groups C and H ($p<0.05$); 0.87±0.03 in group C and 0.97 ±0.02 in group H. Group H had higher ultrafiltration than groups G and I, but there was no statistical significance.

3. Peritonitis episode

The peritonitis episode was highest in group G. There were 0.25episodes/rat/month in group G, 0.14episodes/rat/month in group H, 0.11episodes/rat/month group I.

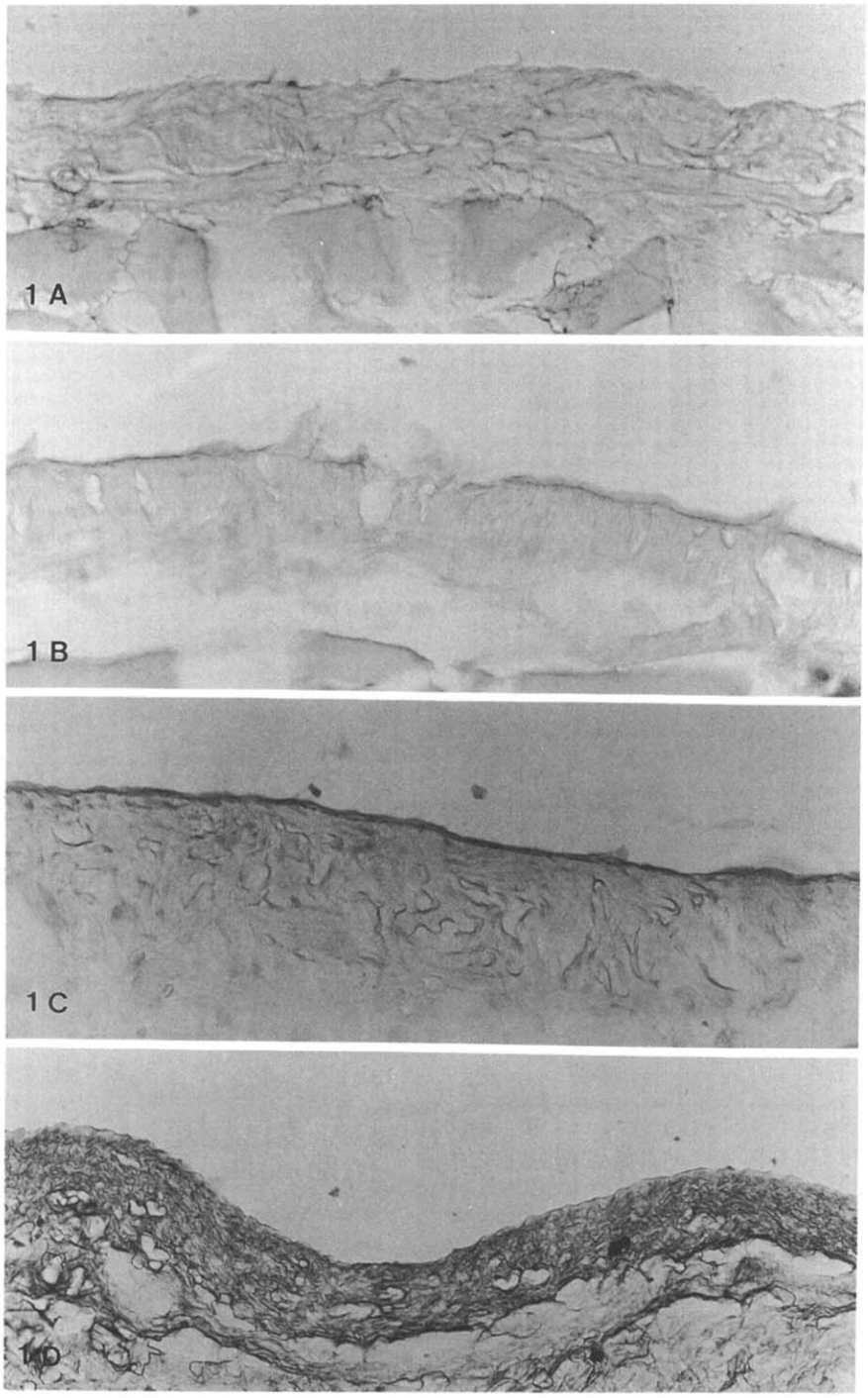

Fig. 1. *Immunohistochemical staining of AGEs in the peritoneal membrane. AGE staining was very minimal or absent in group C (1A), weak in group I (1B), moderate in group H (1C), and strong in group G (1D).*

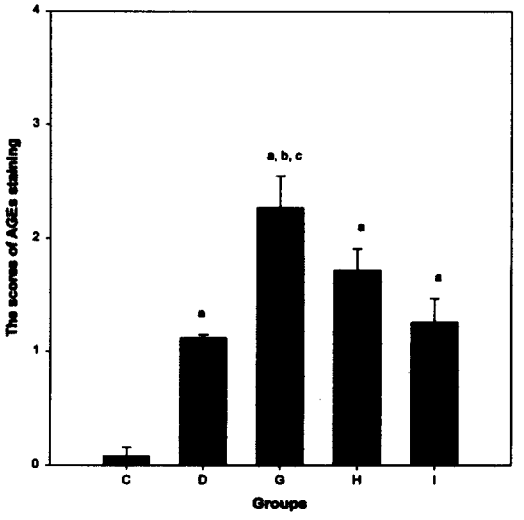

Fig. 2. The scores of AGEs staining in the respective groups. [a]Group C vs p<0.05 in group D, p<0.001 in group G, p<0.001 in gruop H, p=0.008 in group I, [b]p=0.016, group D vs G, [c]p<0.05, group G vs I.

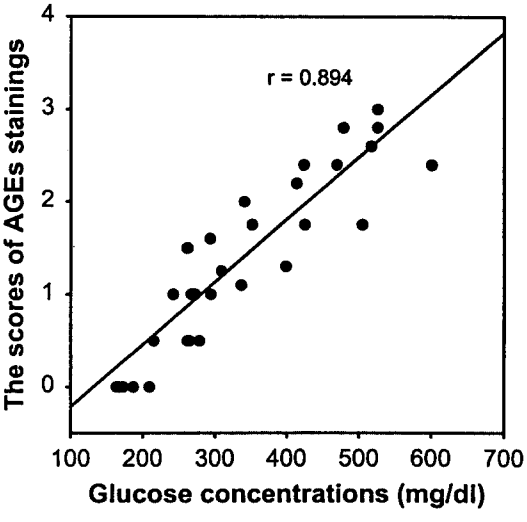

Fig. 2A. Relationship between the scores of AGEs staining and serum glucose concentrations.

Table 1. Changes in D/Do Glucose

Group	1 week	4 weeks	8 weeks	12 weeks
C	0.43±0.03	0.41±0.02	0.40±0.02	0.40±0.01
D	0.42±0.02	0.42±0.01	0.40±0.01	0.39±0.04
G	0.44±0.02	0.41±0.01	0.39±0.01	0.36±0.01
H	0.45±0.01	0.42±0.02	0.38±0.03*	0.35±0.01
I	0.44±0.02	0.43±0.03	0.40±0.02	0.36±0.01

Data are presented as mean±standard error. Abbreviations are; C, intact control rats; D, diabetic control rats; G, diabetic rats dialyzed with glucose solution; H, diabetic rats dialyzed with glucose and icodextrin; I, diabetic rats dialyzed with icodextrin a p<0.05 C vs H

Table 2. Changes in D/P urea

Group	1 week	4 weeks	8 weeks	12 weeks
C	0.83±0.05	0.84±0.03	0.85±0.03	0.87±0.03
D	0.87±0.02	0.88±0.04	0.89±0.04	0.92±0.02
G	0.88±0.02	0.90±0.01	0.92±0.02	0.96±0.04
H	0.88±0.01	0.93±0.03	0.96±0.02*	0.97±0.02
I	0.88±0.02	0.90±0.02	0.93±0.02	0.95±0.01

*Data are presented as mean±standard error. Abbreviations are; C, intact control rats; D, diabetic control rats; G, diabetic rats dialyzed with glucose solution; H, diabetic rats dialyzed with glucose and icodextrin; I, diabetic rats dialyzed with icodextrin, *p<0.05 C vs H*

4. Body weight

Changes in body weight over time are shown in Table 3. The base weight of rats was around 304 grams and the trend showed an increase in weight in all the groups. There were significant differences between group C and groups G, H, I (p=0.001, p<0.001 and p=0.003 respectively at 4 weeks, p<0.001, p<0.001 and p<0.001 respectively at 8 weeks, p<0.001, p=0.001 and p<0.05 at 12 weeks). Diabetic rats (groups D, G, H and I) gained less weight as compared to non-diabetic rats (p<0.05). Control rats (groups C and D) gained more weight than rats on dialysis (groups G, H and I, p<0.05). Rats in group I gained more weight than groups G and H, but there was no statistical significance.

5. Blood tests

Changes in serum glucose levels over time are shown in Table 4. Serum glucose levels were maintained between 250 and 500 mg/dl.

Table 3. Changes in Body Weight (g)

Group	-2 weeks	1 week	4 weeks	8 weeks	12 weeks
C	303.8±2.8	340.0±7.3	396.8± 9.1	422.1±12.2	489.6±13.3
D	304.9±3.8	326.1±4.4	377.1±13.7	387.2±10.9	458.5±14.8
G	303.3±2.9	315.5±7.1	342.2± 8.1*	351.9± 9.2*	408.7± 9.4*
H	302.5±1.9	317.4±6.2	348.0± 6.2†	353.2± 9.3†	415.6±10.3
I	302.8±1.8	314.8±5.9	343.5± 7.4†	349.7± 9.9†	438.7±12.5†

*Data are presented as mean±standard error. Abbreviations are; C, intact control rats; D, diabetic control rats; G, diabetic rats dialyzed with glucose solution; H, diabetic rats dialyzed with glucose and icodextrin; I, diabetic rats dialyzed with icodextrin, *p<0.05 C vs D, †p<0.05 C vs H, †p<0.05 C vs I*

Table 4. Changes in Serum Glucose Level (mg/dl) after Injection of Streptozotocin

Group	1 week	4 weeks	8 weeks	12 weeks
C	149.7± 5.6	151.8 ± 9.8	161.5 ± 9.7	184.7± 8.9
D	276.0± 5.7*	278.6 ±17.3	290.2 ±19.8	286.0±14.8
G	271.9±15.9†	360.9 ±19.6†	487.6 ±57.9†,‖	418.4±45.0†
H	272.4±18.9†	359.13± 9.5†	481.06± 2.4†	384.1±62.7†
I	273.5± 8.9§	330.02± 3.6§	390.1 ±30.8§	345.4±29.4§

*Data are presented as mean±standard error. Abbreviations are; C, intact control rats; D, diabetic control rats; G, diabetic rats dialyzed with glucose solution; H, diabetic rats dialyzed with glucose and icodextrin; I, diabetic rats dialyzed with icodextrin, *p<0.05 C vs D, †p<0.05 C vs G, †p<0.05 C vs H, §p<0.05 C vs I, ‖p<0.05 D vs G*

At one week non-diabetic rats (group C) had lower serum glucose levels than diabetic rats (groups D, G, H and I) ($p<0.001$, $p<0.001$, $p<0.001$ and $p<0.001$, respectively). There were significant differences in serum glucose between group C and groups G, H, I ($p<0.001$, $p<0.001$ and $p<0.05$) at 4 weeks. There were significant differences at 8 weeks between group C and groups G, H, I ($p<0.001$, $p<0.001$ and $p<0.05$), as well as between group D and G ($p<0.05$. At 8 weeks, The serum glucose level in group I was lower than group G and H, but there was no statistical significance. There were significant differences between groups G and C ($p<0.05$) at 12 weeks.

In changes in total cholesterol and triglyceride over time, Group G had higher levels of serum cholesterol as compared to other groups. There were significant differences between groups C and G ($p<0.05$). Group G had higher levels of serum triglycerides as compared to other groups. There were significant differences between group G and groups D and C ($p<0.05$ and $p<0.05$, respectively).

6. Gross findings on termination

In groups C and D the catheters were completely covered by fibrous tissue with severe adhesions, but the abdominal wall and mesentery were clear. In group G, two catheters were completely covered by fibrous tissue with severe adhesions and the other catheters were covered by fibrous tissue with some adhesions. The second catheters in two rats were moderately covered by fibrous tissue as compared to first catheters in the same rats. The abdominal walls of two rats revealed slightly whitish patchy lesions and in one the mesentery revealed slightly whitish patchy lesions.

Two catheters in group H were completely covered by fibrous tissue with severe adhe-

sions, and the other catheters were covered by fibrous tissue with some adhesion. The two second catheters were moderately covered by fibrous tissue as compared to first catheters. The abdominal walls of two rats revealed slightly whitish patchy lesions, but the mesentery was relatively clear.

In group I, one catheter was partially covered by fibrous tissue with severe adhesions, and the other catheters were covered by fibrous tissue with some adhesions. The only second catheter was moderately covered by fibrous tissue as compared to the first catheter. In one rat the abdominal wall revealed slightly whitish patchy lesions, but the mesentery was relatively clear.

7. Histological findings

Collagen deposition in the peritoneal membrane was not seen in groups C and D except one animal in group D that had mild fibrous tissue in the hepatic visceral peritoneum. All sections in group G had mild to moderate fibrous tissues in the hepatic visceral peritoneum and mild fibrosis was noticed in the mesentery of two rats from this group. In group H, 5 of 8 sections had mild to moderate fibrous tissue in the hepatic visceral peritoneum. In group I, 3 of 7 sections had mild fibrous tissue in the hepatic visceral peritoneum.

DISCUSSION

The results of this study showed the following : 1) the AGEs accumulation in the peritoneal tissue was in accordance with concentration of glucose in the peritoneal dialysis fluid. 2) increased permeability to small solutes in rats dialyzed with solution containing glucose. 3) serum glucose levels increased substantially in rats dialyzed with solution containing glucose as compared to rats dialyzed with icodextrin.

Several *in vitro* and *ex vivo* studies have demonstrated that glycosylation and AGEs formation occurs with the use of conventional glucose containing solutions[23, 24]. It has been established that the rate of glycosylation is related to the concentration of glucose and is also dependent on other components of the fluid. An understanding of the chemistry and factors involved in AGEs formation in glucose-based solution has also been established[25]. In this study, The AGEs accumulation was seen in all diabetic rats to varying degrees related to the type of dialysis solutions. The AGEs formation in the icodextrin group was expected to be equivalent to the the AGEs formation in the group D, but the trend showed an inccrease in AGE accumulation in the Goup I as compared to Group D. Even in the control group, one rat showed a small accumulation of AGEs in one section. There is a dearth of information about glycosylation and AGEs formation in the presence of glucose polymers and their final breakdown product maltose under physiological conditions. Recently reported *in vitro* studies have shown a time- and concentration-dependent increase in human serum albumin glycosylation (50 mmol/L phosphate buffer, pH 7.4) with maltose, icodextrin and glucose[26]. The glycosylation rate was comparable for equimolar maltose and icodextrin and was two- to three-fold lower than with equimolar glucose. The glycosylation rate in the standard strength of icodextrin used in peritoneal dialysate (7.5%, 13.2 mmol/L) was more than ten-fold lower than with the lowest glucose concentration used in peritoneal dialysis solution (1.36%, 75 mmol/L). These findings are in accordance with the current study.

The loss of ultrafiltration is typically associated with increased permeability to small solutes such as creatinine[27]. These observa-

tions could be explained if AGEs formation occurred in the peritoneal membrane, resulting in increased permeability to solutes. This might occur via vascular effects or basement membrane thickening, analogous to the situation in diabetes[28]. It is also possible that AGEs modification of collagen in the submesothelial interstitium might diminish the diffusion resistance to small solutes and account for a more pronounced increase in permeability to small solutes than to macromolecules[29]. In the present study, rats dialyzed with glucose-based solution had significantly higher permeability to small solutes such as glucose and urea as compared to control groups, and also a tendency to increased permeability as compared to icodextrin solutions. Cross-linking of basement membrane components by AGEs such as laminin and heparin sulfate proteoglycan or type IV collagen disturbs the integration of the basement membrane[30, 31], which increases vascular permeability. On other hand, the vascular endothelium has AGEs receptors. It is reported that the deposition of AGEs on the receptors induces an increase in permeability of the vascular wall[32]. In the present study, AGEs staining was evident in the vascular walls in all rats except the group C. Accordingly, it is possible that the accumulation of AGEs in the vascular walls of these rats might play a role in the increased peritoneal permeability.

Monitoring the changes in body weight over time is a useful way of assessing the general condition of experimental rats. Our experimental rats gained weight throughout the study. These results suggest that the most of rats were in good condition. Chronic dialysis with glucose solution is associated with various me tabolic disadvantages (especially obesity)[33]. In the present study diabetic rats gained less weight in contrast to non-diabetic rats. Rats on dialysis gained less weight than rats which were not on dialysis. These results suggest that diabetes and dialysis concomitantly induced a stress, which resulted in lesser weight gain.

Prolonged exposure to nonphysiological dialysis solutions resulted in certain inevitable morphologic changes, mainly peritoneal fibrosis[34]. Morphologic analysis of the peritoneal membrane was introduced as a useful way of assessing peritoneal fibrosis[35]. Our results showed that the hyperosmolar glucose solutions group had more episodes of peritonitis. The fibrous tissue covering the catheters of rats dialyzed with glucose solution was thicker and had more adhesion to the abdominal wall as compared to other groups. In addition, whitish fibrotic patchy lesions were comparatively more in rats dialyzed with glucose-based solution. These clinical observations showed that dialysis with higher concentrations of glucose resulted in more peritonitis episodes and was more irritating to the peritoneal membrane, hence resulted in peritoneal fibrosis and catheter blockage.

The maltose and related fractions do not affect glucose metabolisms nor lead to hyperinsulinemia[6]. Furthermore, the lack of hyperglycemia and hyperinsulinemia associated with icodextrin as compared to glucose may offer some long-term metabolic advantages and possibly simplify management of diabetic patients[6]. It is, therefore, possible that this may have a favorable impact on the lipid profile. The cholesterol levels were lower in the icodextrin group as compared to the glucose-based groups in the current study, this did not reach statistical significance. However, the serum glucose levels also increased in icodextrin group in this study, but not to the extent shown in glucose-based group. In a previous study,

Wens et al.[36] demonstrated that the overestimation of glucose in the blood of patients treated with icodextrin is probably related to the presence of oligosaccharides (mainly maltose), derivatives of glucose polymers present in Icodextrin and absorbed via the peritoneal route. A similar finding in this study is in accordance with this hypothesis, and this increase in serum glucose levels in the icodextran group suggests can be attributed to the breakdown products such as maltose.

In conclusion, this study demonstrates that AGEs accumulation in the peritoneal tissues was lower in rats dialyzed with icodextrin as compared to glucose-based solutions. AGEs formation might have a role in the increased permeability of small solutes in PD.

Pharmacological intervention may inhibit or reduce AGEs formation. This may be assessed by using immunocytochemical quantification with validated monoclonal antibodies to AGEs products, with tissue exposed for a sufficient period of time to icodextrin dialysate in experimental animals. Eventually, this may provide insights into new therapeutic strategies for the prevention of deterioration of peritoneal function during PD.

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