Long-term clinical effects of a peritoneal dialysis fluid with less glucose degradation products

BENGT RIPPE, OLE SIMONSEN, OLLE HEIMBURGER, ANDERS CHRISTENSSON, BÖRJE HARALDSSON, GUNNAR STELIN, LARS WEISS, FINN-DAVID NIELSEN, SUSANNE BRO, MICHAEL FRIEDBERG, and ANDERS WIESLANDER

University Hospital of Lund, Lund, Huddinge Hospital, Stockholm, Malmö University Hospital and Sahlgrenska University Hospital, Gothenburg, Länsjukhuset Halmstad, Centralsjukhuset Karlstad, and Centrallasarettet, Borås, Sweden; Rigshospitalet Copenhagen and Hvidovre Hospital, Copenhagen, Denmark; and Gambro AB R & D, Lund, Sweden

Long-term clinical effects of a peritoneal dialysis fluid with less glucose degradation products.

Background. Glucose degradation products (GDPs) are cytotoxic in vitro and potentially toxic in vivo during peritoneal dialysis (PD). We are presenting the results of a two-year randomized clinical trial of a new PD fluid, produced in a two-compartment bag and designed to minimize heat-induced glucose degradation while producing a near neutral pH. The effects of the new fluid over two years of treatment on membrane transport characteristics, ultrafiltration (UF) capacity, and effluent markers of peritoneal membrane integrity were investigated and compared with those obtained during treatment with a standard solution.

Design. A two-group parallel design with 80 continuous ambulatory peritoneal dialysis patients was used. The patients were randomly assigned to either the new fluid (N = 40) or to a conventional one (N = 40), and were stratified with respect to age, diabetes, and time on PD. Peritoneal transport characteristics were assessed by the Personal Dialysis Capacity (PDC™) test at 1, 6, 12, 18, and 24 months after inclusion and by weighing the overnight bag daily. Infusion pain and handling were evaluated using a questionnaire. Peritoneal mesothelial and interstitial integrity were evaluated by analyzing overnight effluent dialysate concentrations of CA 125, hyaluronan (HA), procollagen-1-C-terminal peptide (PICP), and procollagen-3-N-terminal peptide (PIIINP) at 1, 6, 12, 18, and 24 months.

Results. The handling of the new two-compartment bag was considered easy, and there were no indications of increased discomfort with the new system. Furthermore, no changes in peritoneal fluid or solute transport characteristics were observed during the study period for either fluid, and neither were there any differences with regard to peritonitis incidence. However, significantly higher dialysate CA 125 (73 ± 41 vs. 25 ± 18 U/mL), PICP (387 ± 163 vs. 244 ± 81 ng/mL), and PIIINP (50 ± 24 vs. 29 ± 13 ng/mL) and significantly lower concentrations of HA (395 ± 185 vs. 530 ± 298 ng/mL) were observed in the overnight effluent during treatment with the new fluid.

Conclusions. We conclude that the new fluid with a higher pH and less GDPs is safe and easy to use and has no negative effects on either the frequency of peritonitis or peritoneal transport characteristics as compared with conventional ones. Our results indicate that the new solution causes less mesothelial and interstitial damage than conventional ones; that is, it may be considered more biocompatible than a number of conventional PD solutions currently in use.

Conventional fluids for peritoneal dialysis (PD) are potentially bioincompatible because of their hyperosmolality, low pH, and high lactate and glucose concentrations [1, 2]. Especially the high glucose concentrations in conventional fluids have been incriminated as being detrimental to the peritoneum, not the least by providing the substrate for nonenzymatic glycosylation of tissue proteins [2, 3]. Furthermore, during heat sterilization, some of the glucose in conventional solutions is affected by the added heat and converted to fructose through isomerization and degraded to biologically reactive substances [4–7]. Chemically identified glucose degradation products (GDPs) commonly found in PD fluids are methylglyoxal, glyoxal, formaldehyde, 3-deoxyglucosone (3-DG), acetaldehyde, 2-furaldehyde, and 5-hydroxymethyl-furfuralde [6, 7].

These substances have been demonstrated to inhibit cell proliferation and to cause necrosis of fibroblasts, macrophages, and human mesothelial cells in vitro [8–13]. There are indeed indications that GDPs, by causing protein denaturation or cross-linking, are basically cytotoxic [14]. More specific detrimental effects of GDP in PD fluids in vitro include impairment of cytokine release and superoxide radical generation by peritoneal macrophages and by blood mononuclear and polymorphonuclear cells [9, 15]. In animal models, GDPs also seem to cause impairment of leukocyte rolling and an increased

Key words: peritonitis, interstitial damage, dialysate solutions, biocompatible PD solution.

Received for publication December 6, 1999
and in revised form July 10, 2000
Accepted for publication July 25, 2000
© 2001 by the International Society of Nephrology
venular flow velocity [16], even though in acute rat expe-
riments no negative effects of GDP were observed with
respect to peritoneal solute transport or ultrafiltration
(UF) [17]. Clinical studies indicate that GDP, at least in
combination with a low solution pH, may bring about
infusion pain and impaired UF capacity [18, 19].

It has been demonstrated that GDPs are very potent
agents of nonenzymatic cross-linking of proteins, lead-
ning to the formation of advanced glycation end products
(AGEs). In particular, dicarbonyl compounds such as
3-DG and methylglyoxal participate in these reactions
[7, 20–22]. A recent report has demonstrated extremely
high concentrations of 3-DG in conventional PD fluids
[7]. The role of GDP in AGE formation is of great rele-
ance, since AGES are considered to participate in the
remodeling and fibrosis of the peritoneal membrane that
may occur during long-term continuous ambulatory perito-
neal dialysis (CAPD) treatment and that may be linked
to an increased risk of UF failure [23, 24].

To avoid the generation of GDP, the PD fluid may
be sterilized by filtration instead of being autoclaved.
Some generation of GDP will still occur during storage,
however. Another way to avoid the generation of GDP
is to dispense the fluid into a two-compartment bag, where
concentrated glucose is kept at a low pH (in a small com-
partment), separate from the electrolyte-buffer solution
(in a larger compartment). When this system is subjected
to conventional heat sterilization and subsequent stor-
age, the generation of GDP will be very limited. Before
use, the two compartments, however, have to be mixed.
This is the concept of the test solution in the present
study. Toxicological and chemical analyses of a fluid
produced and delivered in this way revealed that, besides
having a pH close to neutral, it showed very low levels
of GDP, essentially below the detection limit for 3-DG,
formaldehyde, and acetaldehyde [25]. With this composi-
tion, the solution’s in vitro cytotoxicity as well as its
ability to induce AGE formation was clearly reduced
[3, 25]. The aim of our current study was to investigate
the long-term impact of this new fluid of PD on perito-
neal solute and fluid transport characteristics, as well
as on some tentative markers of peritoneal membrane
integrity.

METHODS

Study description

The study had a prospective, randomized, parallel de-
sign, including 80 patients randomly assigned to either
Gambrosol 40 CAPD solution [control group (C); N =
40] or to the new, differently produced and nearly neutral
fluid that was low in GDP [study group (S); N = 40].
Both groups were using the Gemini 10 design (with drain
bag) or a slight modification thereof. The concentrations
of GDP, pH, and the in vitro cytotoxicity of the different
fluids are demonstrated in Table 1. To ensure a balanced
composition of the patient material in each group, a
stratified randomization was performed with respect to
patient age (<55, >55 years), diabetes (using insulin or
not), and time on PD (<9 months, >9 months; Table 2).
Initially, the investigation was intended as a one-year
clinical study, but it was later prolonged to include a
study period of two years, without adding any new pa-
ients.

Study population

Patients were recruited if they were at least 18 years
old, gave their fully informed consent, and were able to
use 2 L bags with a calcium concentration of 1.35 mmol/L.
Patients were excluded from the study if they had a
positive screen for hepatitis B or HIV, or had an active
malignancy or were pregnant. Most of the patients (58%)
previously had not been treated on CAPD. Once found
eligible, patients were reported to a central randomiza-
tion office (at Gambro, Lund, Sweden) and were as-
signed to one of the two groups or strata (according to
age, diabetes, and time on PD) using a premade random-
ization schedule. The mean age at entry was 57 years
for the control group and 58 years for the study group.
Diabetic patients represented 43% of the control group
and 35% of the study group, respectively (Table 2).

Study procedure

Patients attended the participating hospital on five
occasions during the study period, that is, at 1, 6, 12, 18,
and 24 months after inclusion. Study parameters were
sampled within two weeks from the scheduled point of
time. Sampling did not take place within an episode of
peritonitis, but was postponed for four weeks after full
recovery from peritoneal infection.

Assessment of peritoneal membrane
exchange characteristics

Personal Dialysis Capacity (PDC™) was used as a tool
to assess the peritoneal transport characteristics [26].
The PDC procedure involves urine, blood, and dialysate
sampling during a standardized CAPD day carried out
according to a special exchange schedule. The program
is based on the three-pore model of peritoneal transport
[27, 28] and describes peritoneal exchange using the fol-
lowing parameters: (I) area parameter (A0/Δx), determin-
ing the diffusion capacity of small solutes, and indirectly,
the hydraulic conductance of the membrane (LpS); (2)
the reabsorption rate of fluid from the peritoneal cavity
to the blood after peak time, when the glucose gradient
has largely dissipated (J,AR); and (3) the large pore fluid
flow (J,L), which determines the loss of proteins to the
PD fluid. Residual renal function (RRF) was also eval-
uated in the PDC procedure and standardized to standard
body surface area (1.73 m²).
Table 1. Chemical and biological characteristics of the two different fluids tested in the clinical study

<table>
<thead>
<tr>
<th>Characteristics (contents of fluid bag)</th>
<th>Control group</th>
<th>Study group</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1.5%</td>
<td>1.5%</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Cytotoxicity %</td>
<td>5.43 ± 0.01</td>
<td>6.43 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>5.30 ± 0.01</td>
<td>6.26 ± 0.01</td>
</tr>
<tr>
<td>UV-abs 288 nm</td>
<td>0.52 ± 0.00</td>
<td>0.13 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>0.83 ± 0.02</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>UV-abs 284 nm</td>
<td>0.20 ± 0.00</td>
<td>0.19 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>0.49 ± 0.01</td>
<td>0.48 ± 0.03</td>
</tr>
<tr>
<td>Acetaldehyde μmol/L</td>
<td>226 ± 5</td>
<td>&lt;1.7</td>
</tr>
<tr>
<td></td>
<td>204 ± 2</td>
<td>≤1.7</td>
</tr>
<tr>
<td>Formaldehyde μmol/L</td>
<td>4.6 ± 0.1</td>
<td>≤2.8</td>
</tr>
<tr>
<td></td>
<td>9.1 ± 0.4</td>
<td>≤2.8</td>
</tr>
<tr>
<td>Methyglyoxal μmol/L</td>
<td>22.7 ± 0.5</td>
<td>5.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>33.3 ± 1.4</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td>Glyoxal μmol/L</td>
<td>5.5 ± 0.3</td>
<td>8.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>7.5 ± 0.1</td>
<td>22.5 ± 1.4</td>
</tr>
<tr>
<td>5-HMF μmol/L</td>
<td>2.2 ± 0.1</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td></td>
<td>6.9 ± 0.1</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>2-Furaldehyde μmol/L</td>
<td>&lt;0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>&lt;0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>3-Deoxyglucosone</td>
<td>118 ± 5</td>
<td>12.3 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>413 ± 19</td>
<td>65 ± 10</td>
</tr>
</tbody>
</table>

Each sample represents the mean value from three separate bags ± SEM. Data are transcribed from references 30 and 11. Abbreviations are: abs, absorption, 5-HMF, 5-hydroxymethyl-furaldehyde.

Table 2. Characteristics of the study patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control group (N = 40)</th>
<th>Study group (N = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)a</td>
<td>57 (26–82)</td>
<td>58 (28–80)</td>
</tr>
<tr>
<td>Malesb</td>
<td>30 (75%)</td>
<td>25 (63%)</td>
</tr>
<tr>
<td>Diabeticsb</td>
<td>17 (43%)</td>
<td>14 (35%)</td>
</tr>
<tr>
<td>Time on CAPDc</td>
<td>0–9 months</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>&gt;9 months</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

a Mean age (range)
b Number of patients (percent)
c Number of patients in different categories (percent)

Ultrafiltration was evaluated during the study by daily registration by the patient of the weight of the overnight bag using an electronic scale. The concentration of glucose in the night bag was 2.5%. Time for start and end of the overnight dwell was recorded, and the dwell time was set at 10 hours.

Dialysate markers

Samples of spent dialysate from the overnight bag were collected and stored without any further treatment and frozen at −70°C for later analysis. Analysis was performed using commercially available kits for CA 125 (enzyme-linked immunosorbent assay (ELISA); Boehringer Mannheim, Mannheim, Germany), hyaluronan (HA; RIA; Pharmacia, Uppsala, Sweden), procollagen-1-C-terminal peptide (PICP), and procollagen-3-N-terminal peptide (PIINP; RIA from Orion Diagnostics, Espoo, Finland). A separate study showed that the level of CA 125 was unaffected by not subjecting the PD fluid to centrifugation before freezing and storage (unpublished observation).

Assessment of infusion pain and handling

The patients completed a questionnaire covering subjective symptoms in connection with infusion of the PD solution. If the answer was yes on the first question of “Do you feel any discomfort or pain during infusion, yes or no?” then the degree of the pain sensation was assessed by a nine-point visual analog scale (VAS).

The breaking of the frangible pin and the mixing of the two compartments of the new bag were each evaluated by four grade scales using scores from 1 to 4 (1, simple; 4, difficult).

Participating centers

The patients were recruited from nine participating centers. The number of study patients (S) and control patients (C) is given within parenthesis: University Hospital of Lund (7 S; 8 C), Malmö University Hospital (4 S; 3 C), Sahlgrenska University Hospital, Gothenburg (12 S; 9 C), Länsjukhuset Halmstad (0 S; 1 C), Centralsjukhuset Karlstad (1 S; 3 C), Centrallasaretett Borås (2 S; 1 C), Huddinge Hospital, Stockholm (3 S; 6 C; all in Sweden), Rigshospitalet Copenhagen (4 S; 1 C), Hvidovre Hospital, Copenhagen (7 S; 8 C; in Denmark). The study protocol was approved by the ethics committee of each participating center.

Statistical analysis

Values are presented as box plots in the figures, with the largest and smallest observed values (if not an outlier or extreme) as bars on the top or at the bottom of the box. The median value is indicated within the box, each box including 50% of the values. Values of more than 1.5 box lengths from the 25th percentile are presented as outliers (o), and values of more than 3 box lengths from the 25th percentile, are presented as extremes (*). The dropout rate as well as the peritonitis incidence (with respect to first episode only) in each treatment group was compared by the log rank test [29]. In addition, the Mann–Whitney test was employed to compare the distribution of the patient-specific numbers of peritonitis episodes during the one-year follow-up. Peritonitis incidence, with respect to all episodes, was calculated and compared according to Rothman [30]. Infusion pain was
Table 3. Distribution of the patients with respect to number of peritonitis episodes

<table>
<thead>
<tr>
<th>Number of episodes</th>
<th>Control group (N = 40)</th>
<th>Study group (N = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

7 dropped out within the first month (4 in the control group and 3 in the study group), and 16 chose (for personal reasons) not to continue a second year. Fifty-two patients completed the first year, and 13 patients completed both years in the study, which represents a dropout rate during the first year of 35% (including the 7 patients who dropped out at the very start) and a dropout rate during the second year of 64% (75%, including the 16 patients who chose not to continue a second year). Except for the 7 initial dropouts and the 16 patients who for various reasons decided not to continue the study, the major dropout causes were transfer to hemodialysis (N = 6), transfer to automatic peritoneal dialysis (APD) or 2.5 L bags (N = 8), transplantation (N = 9), death (N = 7), or various other reasons (N = 14). No significant difference was observed in the dropout frequency between the control group or the study group (P = 0.25; log-rank test).

**Peritonitis**

Table 3 demonstrates the distribution of patients with respect to number of peritonitis episodes. No significant difference with respect to number of episodes or time to the first peritonitis was observed (P = 0.9 and P = 0.35, respectively). The peritonitis incidences were 17 out of 36.41 years (0.467 per year) and 25 out of 45.07 years (0.555 per year) in control and study groups, respectively (P = 0.6). Total peritonitis incidence for the study was 1 out of 23.5 months.

**Peritoneal transport characteristics**

In Figure 2, the normalized “unrestricted pore area over unit diffusion path length” (“area parameter” for small solute transport; A0/x0) is shown as a function of study time in each group. There were no significant differences among control and study groups initially (after 1 month) nor in assessments at months 6, 12, 18, and 24, respectively. This also holds true for the hydraulic conductance (L, S), the final reabsorption rate (J, AR), the large pore fluid flux (J, L), and the renal residual function (data not shown).

**Overnight bag weight**

The overnight bag weight, assessed as a function of treatment time in each group, is shown for consecutive
five-week periods in Figure 3. There were no significant differences whatsoever between the groups \((P = 0.21)\). Moreover, the (patient specific) changes from the “initial” value (mean value of weeks 2 through 5) to each of the following five-week periods did not differ significantly among the groups.

**Dialysate markers**

Figures 4 and 5 demonstrate the levels of CA 125 and HA among the two groups, respectively, at months 1, 6, 12, 18, and 24. CA 125 levels were highly significantly increased for the study group at all points of measurement, whereas HA showed significantly lower values at month 12, and reductions that were of borderline significance at months 1 and 18 \((P = 0.05)\). At 24 months (and partly at 18 months), however, no powerful statistical analysis could be applied because of the low number of patients. The levels of PICP and PIIINP were significantly higher in the test group at months 1, 6, 12, 18, and 24. Thus, for PICP, \(P\) equaled 0.001 at 1 month, \(P < 0.001\) at 6 months, \(P < 0.001\) at 12 months, \(P = 0.02\) at 18 months, and \(P = 0.9\) at 24 months. For PIIINP, \(P\) was 0.002 at 1 month, \(P < 0.001\) at 6 months, \(P < 0.001\) at 12 months, \(P = 0.3\) at 18 months, and \(P = 0.07\) at 24 months. A power calculation demonstrated that a minimum of 14 patients was needed in each group at any time to reveal a 20% significant difference \((P < 0.05; 80\% \text{ power})\).

To use all of the collected data in the statistical evaluation, mean values for all the individual patient marker data for months 1 through 24 were calculated and compared (Table 4). CA 125, PICP, and PIIINP were significantly higher in the study group \((P < 0.001)\), and HA was significantly lower \((P < 0.04)\). The use of individual mean values was justified by the observation that the patient-specific values did not change noticeably during months 1 through 24.

**Infusion pain and handling**

The relative as well as the total number of patients who experienced pain or discomfort during infusion decreased from 31% (21 patients) at 1 month and 33% (19 patients) at 6 months to 13% (6 patients) at 24 months. Since a sufficient number of patients reported pain at one and six months, these data were chosen for further evaluation. At one month of the study, 40% (12 of 30) of the patients on conventional fluid and 24% (9 of 37) of the patients on the new fluid noted some kind of pain or discomfort. After six months, these figures were 46% (11 of 24) and 24% (8 of 34), respectively. Although the new fluid tended to yield fewer patients with discomfort or pain, this was not found to be statistically significant \((P = 0.19\) and \(P = 0.09,\) respectively, using Fisher’s exact test for the dichotomous pain variable). For the patients reporting pain, no significant differences were seen in the distribution of the degree of pain using the nine-point scale rate (at 1 month \(P = 0.12\), at 6 months \(P = 0.13,\) Mann–Whitney test).

Evaluation of the study questionnaire indicated that the handling of the new bag was easy. On a four-grade scale with scores from 1 to 4 (1, simple; 4, difficult), the new fluid scored a mean value (declining over time)

---

**Fig. 2.** Box plot of normalized (%) area parameter \(A_0/\Delta x\) as a function of treatment time in the \((A)\) control group and \((B)\) study group. Each box includes 50% of all measured values, with the largest and smallest observed value as bars on the box top or at the box bottom and the median value indicated within the box. Values of more than 1.5 box lengths from the 25th percentile are presented as outliers (o), and values of more than three box lengths from the 25th percentile are presented as extremes (*). \(N\) is the number of patients. There were no significant differences among study and control groups at any time with respect to \(A_0/\Delta x\).
Fig. 3. Box plots of drained weight in the overnight bag (2.5% glucose concentration) as a function of treatment time in the (A) control and (B) study groups. $N$ is the number of patients. A patient had a valid weekly observation, if at least one value was reported during each week. The number of valid observations decreased more markedly in the control group than in the study group. No statistical difference among control and study groups was obtained.

Fig. 4. Box plot of the concentration of CA 125 (U/mL) in overnight peritoneal effluent for the (A) control and (B) study groups. The study group showed significantly higher values at all points of measurement ($P < 0.001$ at 1 month, $P < 0.001$ at 6 months, $P = 0.001$ at 12 months, $P = 0.003$ at 18 months, and $P = 0.007$ at 24 months).
Fig. 5. Box plot of the concentration of hyaluronan (HA) (ng/mL) in overnight peritoneal effluent for the (A) control and (B) study groups. The study group showed lower (or marginally lower) values at 1, 12, and 18 months. \( P < 0.05 \) at 1 month, \( P = 0.6 \) at 6 months, \( P = 0.04 \) at 12 months, \( P = 0.05 \) at 18 months, and \( P = 0.26 \) at 24 months). Overall, the HA concentration was lower in the study group (Table 4).

Table 4. Dialysate concentration of CA 125, HA, PICP and PIIINP

<table>
<thead>
<tr>
<th>Dialysate marker</th>
<th>Control group</th>
<th>Study group</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 125 U/mL</td>
<td>25 ± 18</td>
<td>73 ± 41</td>
</tr>
<tr>
<td>HA ng/mL</td>
<td>530 ± 298</td>
<td>395 ± 185</td>
</tr>
<tr>
<td>PICP ng/mL</td>
<td>244 ± 81</td>
<td>387 ± 163</td>
</tr>
<tr>
<td>PIIINP ng/mL</td>
<td>29 ± 13</td>
<td>50 ± 24</td>
</tr>
</tbody>
</table>

The individual patient mean values for months 1 to 24 were calculated for each treatment group. Data are shown as mean ± SD. CA 125, PICP and PIIINP values were higher in the study group \( P < 0.001 \) and HA was lower \( P < 0.04 \).

between 1.1 and 1.4 (frangible pin) and 1.0 to 1.2 (fluid mixing). The study represents approximately 257 treatment months and close to 65,000 exchanges with the new bag. Thus, the handling of the new bag can be considered easy and safe.

Subgroup analyses

Subgroup analyses did not reveal any differences among diabetics versus nondiabetics or among newly recruited patients versus patients who had been on PD previously with regard to any of the parameters discussed previously in this article. Among the first-year dropouts, 21 patients completed at least the first month of the study, and of these, 13 belonged to the control group and 8 to the study group. In neither group the dropouts differed significantly with respect to \( A_0/Ax \) (transport type) or CA 125 dialysate level from the non-dropouts of the corresponding group.

DISCUSSION

The present study supports and extends the results of a previous preliminary crossover study [31], demonstrating that a new glucose-based solution of more or less identical composition as conventional ones, but having a near neutral pH and being largely free of GDP, significantly affects some effluent markers of peritoneal membrane integrity. Thus, CA 125, PICP, and PIIINP were found to be significantly higher and HA lower with the new fluid after one month and during up to 18 months of treatment. However, there were no measurable effects on peritoneal exchange parameters, including UF, with either the new or with the conventional control solution during the two years of observation. A much longer study period would have been required to reveal any changes in these parameters, since substantial increases in small solute (glucose) transport and reductions in UF tend to first appear—if at all—after three or four years of treatment using conventional solutions [32].

Mesothelial cells lining the entire peritoneum produce lubricants and surface tension-lowering substances, such as phospholipids, but also fibrinolytic and antithrombotic substances, as well as cytokines and chemokines, and a number of other compounds, including HA, CA 125, and collagen peptides [33, 34]. In this context, CA 125 and phospholipids can be regarded as more or less specific for the mesothelium, whereas the other compounds also are produced by other cells in the peritoneum. In fact, CA 125 effluent levels have been claimed to reflect the mass of viable mesothelial cells in stable PD patients [35–37]. During PD, the mesothelial cells usually become cubic and multinucleate, tend to lose their microvilli, and express more rough endoplasmatic reticulum, at least over longer observation periods [38]. The expression of more rough endoplasmatic reticulum should, at least partly, reflect an increased mesothelial metabolic activity in response to the continuous loss of
secretion products to the dialysate that occurs in PD [33]. Thus, the mesothelium is apparently ‘‘up-regulated’’ to produce more secretion during CAPD in response to the repeated exchanges of dialysate. Conceivably, up-regulation of the mesothelium in this respect would be more pronounced for a more physiological solution than for a conventional one. Hence, the production of phospholipids, CA 125, collagen peptides, etc., can be expected to be higher with the new, more physiological PD fluid. On the other hand, inflammatory stimulation of the mesothelium using proinflammatory cytokines has not been found to affect the release of CA 125 above baseline levels, in contrast to the situation for HA (discussed later in this article) [35].

Mesothelial cells are frequently lost during the course of CAPD. Thus, along with an impaired remesothelialization, a frequent finding in long-term PD, partial mesothelial denudation occurs [38]. Impaired survival of mesothelial cells might thus serve as an early indication of a more or less severe peritoneal tissue damage or irritation, eventually terminating in fibrosis. Such changes seem to occur during long-term treatment concomitant with microvascular alterations, such as capillary neovascularization, and possibly, increases in vascular permeability. Compared with the capillary barrier, mesothelial cells and interstitial tissue are, however, unlikely to play an important role in the peritoneal transport of fluid and solutes [2, 27, 28, 36, 39]. Thus, early mesothelial and interstitial changes would not be reflected by alterations in peritoneal transport parameters [36].

Longitudinal examination of the dialysate during the course of CAPD, in accordance with the morphological changes, has demonstrated declining CA 125 dialysate levels with increasing treatment time [37]. Our results of higher dialysate CA 125 levels during the use of the new fluid strongly indicate that the mesothelial cells are not as negatively affected throughout the treatment as by conventional PD fluids. Elevations of dialysate in CA 125 were observed early, that is, after a treatment period of only one month [40], which was similar to observations in our previous cross-over study [31]. Here, significant increases in CA 125 were observed as soon as 6 and 12 weeks of treatment for a solution largely free of GDP. Furthermore, the CA 125 levels again dropped to low values after switching the patients back to conventional dialysis fluids [31, 40]. Whether these changes reflect alterations of the mesothelial cell mass, or alternatively, were due to changes in CA 125 production per cell is not known. One should keep in mind that approximately 40% of our patients had been previously exposed to conventional PD fluids. This group of patients also showed an elevation of CA 125 during treatment with the new fluid; that is, they did not differ from the group of patients who had never been exposed to PD fluids before. Thus, if mesothelial cell mass is positively influenced by the new fluid, the rise in CA 125 may reflect a rapid regeneration of the mesothelium during treatment with the new solution. Actually, ex vivo studies have recently demonstrated that mesothelial cells (from the overnight effluent) grow to confluence in culture in a shorter time when harvested from patients who have been on PD fluids with less GDP than when harvested from patients previously exposed to conventional solutions (abstract; Järkelid et al, Perit Dial Int 19:S54, 1999). These findings indicate that the mesothelial cells seem more viable when exposed to the new fluid. Hyaluronan is a major component of the interstitial ground substance in most tissues. It is secreted into the peritoneal cavity by fibroblasts in the interstitium and locally by the mesothelial cells [41]. HA is involved in several physiological processes, such as tissue repair and remodeling, and also in primary morphogenesis [42]. The local HA production by tissue fibroblasts (and by the mesothelium) increases very markedly, for example, during peritonitis [41]. On the other hand, in edema states or during conditions of high fluid flows through an uninflamed interstitium, HA is easily washed out from the tissue via the lymphatics [43]. The fraction of HA that is produced by mesothelial cells forms a mesothelial surface coat together with other glycosaminoglycans (GAGs) and phospholipids [33, 42]. The continuous ‘‘rinsing’’ of the peritoneal membrane by conventional PD solutions most likely causes mesothelial HA production to increase slightly, since the concentration of HA is known to be considerably lower in fluid obtained at the first washout in patients entering CAPD [41]. However, the marked increases in HA production by both fibroblasts and mesothelial cells that occurs in peritonitis reflect inflammatory stimulation. In fact, HA seems to act as a peripheral inflammation marker [42]. In this perspective, the lower HA production in the test group is consistent with a more physiological situation for the new solution.

Procollagen-1-C-terminal peptide and PIIINP are released during the synthesis of collagen I and collagen III, respectively, and are used as markers for overall collagen synthesis [33, 34, 44]. Dialysate concentrations reflect locally produced PICP and PIIINP by fibroblasts and mesothelial cells [33, 44]. Our results of a higher concentration of PICP and PIIINP for the new fluid most probably reflect an improved cell function of the mesothelium and thus more collagen release to the effluent. The data may, of course, also indicate an ongoing fibrosis with a higher collagen turnover. In this context, however, it is noticeable that Graff et al did not show a correlation between the production of collagen peptides and long-term CAPD or peritonitis incidence, conditions that would be expected to be associated with more peritoneal fibrosis [44].

The mechanism by which GDP alone or in combination with high glucose concentrations (and other toxic influences) affects the mesothelial cells remains to be
elucidated. One possibility is that there is a direct toxic action of GDP through carbonyl-induced cross-linking of surface proteins and of intracellular proteins or nucleic acids. For instance, formaldehyde, a well-known fixative, easily penetrates the cell membrane and immediately cross-links several intracellular components. Furthermore, GDP can promote AGE formation in an environment of high glucose levels, which is of great relevance since AGE seem to reduce the viability of mesothelial cells [45, 46]. It is conceivable that the major detrimental action of GDP over longer periods of PD treatment is linked to such a promotion of AGE formation in the presence of high glucose levels [23, 46].

One cannot rule out the possibility that the higher pH in the new fluid is a major factor in improving its biocompatibility, at least in the short term. Actually, in a recent rat study, GDP-free solutions seemed to reduce the initial peritoneal vasodilatation significantly, and hence, the initial inflation of peritoneal glucose transport produced by conventional (acidic) solutions. However, this occurred only when the GDP-free solutions were buffered to a neutral pH. Indeed, it was recently shown that a neutral bicarbonate (25 mmol/L)/lactate (15 mmol/L)-buffered PD solution reduced inflow pain compared with conventional (acidic) control lactate solutions [47]. In fact, the combination of bicarbonate/lactate was more effective than bicarbonate (38 mmol/L) alone in reducing infusion pain. This may be ascribed to the usually high extracellular pCO₂ produced when the intraperitoneal bicarbonate concentration is >30 mmol/L and there is a subsequent rapid diffusion of CO₂ intracellularly, causing intracellular acidosis [48, 49].

We conclude that a new PD fluid with a higher pH and less GDP than conventional ones is safe and easy to use, with no negative effects on either the frequency of peritonitis or on peritoneal membrane transport characteristics. Moreover, it turns out to be more biocompatible for the patient, since according to all available evidence, it tended to cause less mesothelial and interstitial damage, although peritoneal microvascular function appeared to be unaffected. In the long term, the use of this solution rather than conventional dialysate formulas will hopefully lead to a more intact peritoneal membrane with better preserved transport characteristics.

ACKNOWLEDGMENTS

This study was supported by Gambro Sweden AB and also in part by funding from the Swedish Medical Research Council (grant no. 8285). The support and assistance from the following persons are gratefully acknowledged: Gerd Hallgren and Susanne Zetterman (Centrallasaretet Borås, Sweden), Peter Johansson (Lundsjukhuset Halmstad, Sweden), Ann Berglund-Back (Centraljukhuset Karlstad, Sweden), Vreni Frölich (Kungsholmsdialyzen Stockholm, Sweden), Elisabeth Hildebrand, Lene Möller (Rigshospitalet Copenhagen, Denmark), Pia Trojahn (Hvidovre Hospital, Denmark), Lena Krutzen (University Hospital of Lund, Sweden), Ann-Britt Graham and Elisabeth Risansen (Malmo University Hospital, Sweden), Ellinor Broms (Sahlgrenska University Hospital Gothenburg, Sweden), and Christina Landgren, Christina Schönborg, Anette Larsen, Helen Andersson, Annika Larsson, Helle Larsson, Thierry Crost, Eva Svensson, Lena Järkélid, Per Kjellstrand, Mary-Ann Ernstsson, and Lena Ohsson (Gambro AB). We also thank Ulf Strömbäck for statistical analysis and Kerstin Wihlborg for skilful typing of the manuscript.

Reprint requests to Dr. Bengt Rippe, Department of Nephrology, University Hospital of Lund, S-221 85 Lund, Sweden.
E-mail: Bengt.Rippe@njur.lu.se

REFERENCES

19. LAMB EJ, CATTELL WR, DAWNAY AB, et al: In vitro formation of...


