

The Mesothelium Under the Siege of Dialysis Solutions: Old Glucose, New Glucose, and Glucose-free Osmotic Agents

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Use of one bag of glucose peritoneal dialysis fluid (PDF) results in the development of a dose-related senescent mesothelial cell phenotype not linked to acidic pH or the presence of lactate buffer. This complication derives mostly from oxidative stress induced both by glucose itself and by glucose degradation products (GDPs).

New glucose formulations are offered in dual- or three-chambered bags, keeping the glucose at a low pH. The result is a reduced presence but not complete elimination of GDPs. These formulations have the potential to slow injury to the peritoneal membrane. Icodextrin and amino-acid PDFs, used for one exchange daily, have advantages and drawbacks alike. Icodextrin offers excellent ultrafiltration, but on the other hand, mesothelial cells incubated with this osmotic agent show reduced viability and proliferation and DNA damage. These unwanted effects appear to result from a substantial degree of oxidative stress. An amino-acid-based PDF offers a positive nutritional effect; however, its ultrafiltration capability is not higher than an equimolar 1.5% glucose solution. Amino-acid solution appears to be more biocompatible than glucose-based fluid.

Sofar, glucose PDF offered in a single-compartment bag is not a biocompatible solution for long-term peritoneal dialysis. Icodextrin does not appear to be more biocompatible than glucose. Amino-acid-based solution is less harmful to the mesothelium, but its usefulness is still limited.

Key words

Osmotic agents, glucose, icodextrin, amino acids

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Introduction

The chemical composition of dialysis solutions has been a matter of controversy since the early days of clinical peritoneal dialysis. Since the late 1950s, glucose-enriched, lactate-buffered, single-compartment solutions have been widely used for acute and long-term maintenance peritoneal dialysis. Research reported during the 1980s delivered new glucose-free formulations using icodextrin or amino acids. Because the use of the foregoing osmotic agents is limited to 1 exchange daily, glucose-based solutions are still required. More recent research resulted in the design of dual- or three-chambered bags to minimize the formation of glucose degradation products (GDPs) during the process of heat sterilization.

The present review analyzes the tissue biocompatibility of the above-mentioned peritoneal dialysis fluid (PDF) formulations with regard to the mesothelial monolayer and tries to answer the question of whether glucose may still be a valuable osmotic agent.

Discussion

The classic single-compartment, lactate-buffered, low-pH, glucose-enriched PDFs

THE CASE AGAINST "OLD GLUCOSE"

A large body of literature supports the contention that the biocompatibility of the classical PDF formulation is quite limited.

In vivo, 2 hours' exposure to fluid with a high glucose concentration is enough to induce a marked acceleration of the life cycle of the exposed mesothelial cell population. At the same time, cell viability is substantially reduced, and signs of osmotic stress become evident (1). Long-term exposure to the dialysis solution results in the development of a dose-related

senescent mesothelial cell phenotype: changes were more marked with the use of a 4.25% PDF as compared with a 1.5% solution. On the other hand, differences were not dependent on pH, because observations at pH 5.2 were not significantly different from those made when the monolayer was exposed to solution at pH 7.0. In addition, a similar senescent mesothelial phenotype was observed *in vivo*, after long-term exposure to 4.25% glucose-enriched, filter-sterilized fluid prepared in Hank's balanced saline solution (BSS) at pH 7.0 (2).

It has been shown that glucose *per se* has a specific effect on the outbreak of oxidative stress. Experiments performed *in vitro* unveiled the fact that mesothelial cells in culture generate substantial amounts of hydrogen peroxide when exposed to high glucose concentrations. This phenomenon is most likely linked to damage to the mitochondrial DNA observed both in cultured mesothelial cells and in the monolayer in patients on long-term continuous ambulatory peritoneal dialysis (3).

The observed acceleration of the cell's life cycle from a degree of acute oxidative stress induced both by glucose itself and by GDPs derives from the nonenzymatic degradation of glucose. Glucose degradation products retard remesothelialization independently of D-glucose concentration (4).

Long-term exposure to dialysis solutions of this kind affects all components of the peritoneal membrane. Mesothelial cells dressing the cavitory aspect of the peritoneum show a phenotype substantially different from that seen in intact, unexposed animals; the population is furnished mostly with large multinucleated senescent cells, showing low-density distribution and a significantly reduced degree of viability. This failing regeneration capability becomes critical in clinical peritoneal dialysis, because the procedure is linked to a process of continuous injury coupled with also-continuous regeneration. A reduced regenerative capability also results in the development of depopulated areas in which fibrous tissue replaces the absent monolayer (5).

Experimental observations support the contention that injury to the mesothelial monolayer by means of oxidative stress appears to be a relevant mechanism leading to peritoneal sclerosis (6). Furthermore, glucose and its degradation products are at the origin of the generation and deposition in the peritoneal membrane of advanced glycation end-products

(AGEs), whose accumulation is associated with markedly altered peritoneal permeability (7).

At the ultrastructural level, use of standard glucose solution for long-term peritoneal dialysis in nondiabetic patients resulted in duplication both of the submesothelial and the capillary subendothelial basement membrane (8). Similar results were detected in laboratory animals exposed for several weeks to a glucose-enriched solution. In addition, perfusion studies using the cationic tracer ruthenium red showed that 3 months' exposure of rat peritoneal cavity to high glucose PDF results in a significant reduction of the electronegative charges normally present under the submesothelial basement membrane, which in turn leads to increased peritoneal permeability to albumin (6).

The reaction of mesothelial cells to hyperosmolar high glucose concentrations was first investigated by Breborowicz *et al.* (9), who detected substantial shrinkage of mesothelial cells in culture after as little as a 30-minute exposure to 180 mmol/L glucose. Similar observations were made using mannitol in the same molar concentration. Used for longer periods of time (7 days), each osmotic agent resulted in regulatory volume increase, suggesting a kind of adaptation to the hyperosmolar environment. Observations made in rats (10) also showed an enlarged cytoplasmic surface area as a sign of regulatory volume increase after 15 and 30 days' exposure both to glucose and to mannitol PDF in equimolar concentrations (approximately 486 mOsm/L). In both cases, reversal to normal values was observed after a recovery period of 30 days. However, the viability of the cells was significantly lower in animals exposed to high glucose. This important difference may be linked to the fact that osmotic stress results in oxidative injury (11); mannitol, which does not permeate the cell membrane, is a known hydroxyl radical scavenger.

Numerous *in vitro* studies have showed that the combination of lactate buffer and low pH (pH 5.2 – 5.5) has detrimental effects on various cell lines in culture, including mesothelial cells. It has also been shown that exposure of cells to lactate buffer, but with a pH of 7.0, prevents most of the changes detected when using the acidic solution (12). However, the interpretation of *in vitro* studies and their extrapolation to the *in vivo* situation is quite problematic. The best *in vitro* system fails to exactly reproduce the continuous changes that characterize the

in vivo experimental and clinical situations. The aforementioned studies were short-term acute *in vitro* experiments designed to analyze the effects of a complete multi-ingredient solution whose chemical composition is far from that of the extracellular fluid of living mammals. Furthermore, to evaluate biocompatibility, the analysis looked at the reactions of cells dissociated from their natural three-dimensional geometry when exposed to the physicochemical challenge of the experimental solutions.

In vivo, studies in rats showed that 1 daily injection of glucose-free, lactated-buffered solution at pH 5.2 performed over a period of 30 consecutive days failed to induce significant changes in density distribution, mean cell size, mean cytoplasmic surface area, prevalence of large senescent cells, multinucleation, mitotic activity, and cell viability in the exposed monolayer (13). From this experiment, it may therefore be concluded that lactate buffer is not behind the development of the aforementioned hypertrophic, senescent aspect of the mesothelium, which consequently can be concluded to result from long-term exposure to glucose. That assertion is supported by biocompatibility studies of PDFs buffered with lactate, but with a pH of approximately 7.0 – 7.4, quite close to the normal cytosolic pH of 7.2, suggesting that lactate is not less biocompatible than the new bicarbonate-based solutions (14).

Low pH is one more controversial issue. At this point, it should be mentioned that, after a dwell time of 15 minutes, dialysate pH is around 7.0, a level of acidity quite close to the intracellular physiologic pH. Equilibration is reached after a 30-minute exposure. Here again, there is disagreement between *in vivo* and *in vitro* studies. Low intracellular pH resulting in reduced antibacterial capability has been reported in leukocytes incubated in acidic glucose PDF (15). On the other hand, *in vivo* experiments performed in mice have shown that pH has no role in the development of the hypertrophic, senescent mesothelial phenotype derived from use of high glucose in the classical PDF (1) and in the 4.25% glucose concentration in Hank's BSS at pH 7.3 – 7.4 (2). More recent *in vivo* investigations have shown that the biocompatibility of an acidic or neutral lactate–bicarbonate solution is unrelated to pH (16).

Up to this point, it appears evident that “old glucose” did its job. Because of the poor biocompatibility uncovered during many years of research, new efforts

were required to circumvent its shortcomings—that is, the high concentrations of GDPs, and not less important, the still unsolved problem of oxidative stress.

THE NEW GLUCOSE-ENRICHED SOLUTIONS

The “new glucose” formulations share the technical improvement of being offered in dual- or three-chambered bags, to keep glucose at low pH (approximately 3.0 – 3.2) until the very moment of starting a dialysis session. Use of the dual-compartment system reduces the presence of GDPs, but does not completely eliminate them, and makes possible the manufacture of bicarbonate-buffered PDF free of the risk of calcium carbonate precipitation. The final pH of these solutions after mixing ranges between 6.6 and 7.4 [bicarbonate-buffered Bicavera (Fresenius Medical Care, Bad Homburg, Germany): 7.4; bicarbonate/lactate–buffered Physioneal (Baxter Healthcare Corporation, Deerfield, IL, U.S.A.): 7.4; lactate-buffered Balance (Fresenius): 6.8 – 6.9; lactated-buffered Gambrosol Trio (Gambro Lundia AB, Lund, Sweden): 6.6], differences that do not seem to be relevant (16).

In vitro studies have shown that, in mesothelial cells incubated in a low-GDP-containing solution, viability and proliferation are improved (17). In addition, observations made in the clinic point to better biocompatibility of the new low-GDP solutions as compared with the classical single-compartment glucose-based fluid (18).

So far, *in vitro* and *in vivo* studies have shown that, in spite of the differences in pH and buffer composition, all the “new glucose” formulations show the potential to slow the injury to the peritoneal membrane associated with the use of conventional single-compartment solution.

The new osmotic agents

ICODEXTRIN

Icodextrin-based solution has been defined as biocompatible agent because of its low osmolarity (284 mOsm/L) and low GDP content (19), expected to minimize generation of AGEs in the exposed membrane. These concepts are derived mainly from *in vitro* experiments (19) that resulted in better fibroblast proliferation and reduced formation of albumin AGEs than were observed with glucose. However, AGE formation in collagen was not significantly different

after incubation with icodextrin or with glucose (20). Conversely, conflicting observations were seen in other experiments. Incubation of human peritoneal mesothelial cells (HPMCs) in icodextrin resulted not only in reduced viability, proliferation, mitochondrial aerobic metabolism, and cytokine production, but also in DNA damage—effects that were also detected *in vivo* (21). Moreover, icodextrin and glucose both retard mesothelial cell repopulation after experimentally induced injury, leading to development of peritoneal fibrosis through repair using connective tissue and the consequent development of peritoneal sclerosis (22).

All these unwanted effects of icodextrin appear to result from a substantial degree of oxidative stress (23). Investigations performed in patients substantiate the concept that carbonyl stress compounds (RCOs) are present in effluent of patients treated with icodextrin (24). This increase in RCOs may therefore well be the source of the persistent peritoneal inflammatory reaction associated with the use of icodextrin detected in clinical use (25). The eventual deposition of AGEs on peritoneal tissues resulting from exposure to icodextrin cannot be elucidated clinically, because PD prescriptions typically combine one bag of icodextrin with three or more of glucose-enriched solution.

Thus far, taking into consideration the accumulated information, it may be concluded that the 7.5% icodextrin solution does not seem to be more biocompatible than single-compartment lactated glucose PDF.

AMINO ACIDS

Amino acids as an alternative osmotic agent were introduced to improve the nutrition status of peritoneal dialysis patients. The positive nutritional effect of amino-acid PDF is somewhat counterbalanced by its modest ultrafiltration capability, not higher than equimolar 1.5% glucose. In terms of biocompatibility, *in vitro* studies have shown that the oxidative metabolism and phagocytic and opsonic activity of peritoneal macrophages exposed to a 1% solution of amino acids at neutral pH are less affected by that fluid than by glucose-based solutions (26).

Published evidence indicates that mesothelial cells express eNOS (nitric oxide synthase), and consequently have the capability to generate NO (27). This outcome is stimulated when cells are incubated in

amino-acid solution. In patients on long-term peritoneal dialysis, eNOS expression increases by a factor of 5 in peritoneal tissue. It has been postulated that increased eNOS expression is one more factor leading to peritoneal angiogenesis, because NO is necessary for the biologic activity of vascular endothelial growth factor (28). In spite of this open question, it may be concluded that PDF with amino acids is more biocompatible than the conventional glucose- or icodextrin-based solutions, even though this advantage may be counterbalanced by its still-debated, more modest ultrafiltration capability as compared with that of almost equimolar glucose-based PDF.

Conclusions

After a long journey, it seems evident that “old glucose” PDF, offered in a single-compartment bag, is not a biocompatible solution for long-term peritoneal dialysis. In turn, icodextrin does not appear to be more biocompatible. Amino-acid-based solution is less harmful to the mesothelium, but its use is still limited.

Use of the new multi-chambered low-GDP formulations brings about a remarkable biologic improvement in terms of damage to the monolayer. However, even though the magnitude of oxidative injury from glucose itself or its degradation products (or both) has been substantially reduced, it has not yet been eliminated. Consequently, and looking to the future, additional research aimed at the inclusion of biologic antioxidants in the improved new formulations would be welcomed.

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