The un-physiology of peritoneal dialysis solution and the peritoneal membrane: from basic research to clinical nephrology

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La Greca: Peritoneal dialysis (PD), haemodialysis, and transplantation are the possible modes of renal replacement therapy and I have been convinced for many years that all three should be offered to ESRD patients as an integrated set of treatment modalities.

Recent studies comparing PD and haemodialysis patients demonstrated that both are equally effective, and that results within 5 years of starting replacement therapy in terms of survival, rehabilitation, and quality of life were comparable.

So, in agreement with Coles and Williams [1], I believe that in the absence of absolute contraindications, PD is the first choice for ESRD patients because it is less expensive than HD and, being home treatment, does not require an excessive increase in the number of dialysis units. PD moreover represents an excellent ‘parking area’ for patients awaiting transplantation. Therefore, every effort must be made to preserve the integrity and function of the peritoneal membrane for as long as possible.

According to many authors, the peritoneal membrane, especially the basement membrane below the mesothelium, undergoes diabetic-like modifications. Is this statement true?

Coles: What we actually have is a situation where although the thickness of the interstitium is obviously increasing, we also have an increasingly small solute transport. In other words, the interstitium cannot be acting as much of a barrier to small solutes. The other thing is that, although there is an association with increased small solute transport and increased protein loss, this is not an enormous protein loss. The problem is the water rather than the small solutes, because while we don’t lose solute clearance, apparently we lose water transport. So I would personally vote that this is a diabetic-like situation, but not necessarily exactly the same as diabetes.

Noonan: That the pathology of diabetic nephropathy and the loss of filtration in the peritoneum with dialysis are related to similar problems is a frequent axiom. This includes basement membrane thickening and the effects of advanced glycation end-products (AGEs) on tissue function. However, from a molecular point of view, the glomerulus and the peritoneum probably have very different compositions. We have to remember that the glomerular basement membrane (GBM) is a unique basement membrane; its major collagen IV chains are z3, z4 and z5, while those of the peritoneum are likely to be z1 and z2. The laminins in the GBM are mostly laminin 5 and 11, while I suspect they are different in the peritoneal basement membrane. The major proteoglycan of the GBM is agrin, while that of most other basement membranes is perlecan. So the basement membranes we are looking at are actually completely different molecular structures. Finally, Karl Tryggvason’s group (Kestila et al.) [2] recently cloned the gene for Finnish type congenital nephrosis, where there is massive urinary protein loss. This gene encodes a protein called nephrin, that is localized in the slit diaphragm, not the GBM. The slit diaphragm appears to be a structure unique to the glomerulus and to play a key role in filtration. This has opened many questions as to which structures are responsible for the filtration function in the glomerulus, and how these are affected in diabetes. Thus, can we really compare diabetic nephropathy to peritoneal filtration dysfunction in dialysis?

Schleicher: In reference to the axiom that AGEs affect both the glomerular and the peritoneal membranes, and this establishes a similarity between diabetic nephropathy and the loss of filtration in the peritoneum with dialysis, I have some concern. By measuring the glycation of extracellular matrices and namely of the GBM, the increase in glycation is less than expected compared with the extent of the hyperglycaemia of the patients [3]. This is because the collagen turnover in basement
membranes is higher in diabetics due to an increased synthesis, which causes a lower than expected glycation [3]. In any case, absolute glycation is rather low; it is about 1–3% covering the normal glucose concentration and the whole diabetic range, i.e. only 1–3% of all lysine residues of the collagen of the GBM are glycated. Based on these results, I do not really suspect that the glycation or the AGE content of basement membranes per se may be really very important, unless the AGE content of the peritoneal membrane is much higher than in other basement membranes. Unfortunately this is not known.

Noonan: In reference to this point, we should consider the role of the oxidative stress induced by AGEs, since it seems to be very important in the damage induced by glycation, for instance apoptosis. What is the level of apoptosis in these tissues when exposed to high glucose?

Amore: We recently demonstrated that Amadori products1 of glycated albumin, a reactive intermediate of the Maillard reaction obtained by incubating native human albumin with commercial PD solutions, induced a significant increase in the apoptotic rate of cultured mesothelial cells [4]. The same effect was observed when mesothelial cells were incubated with dialysates obtained after 12 h dwelling time in long-term PD patients [5]. Increased apoptosis occurs in parallel with an increased synthesis of the inducible isoform of nitric oxide (NO) synthase, and can be inhibited by co-incubation with the specific agonist of NO, L-nitromethylarginine, suggesting the role of NO. The same phenomenon was also demonstrated in endothelial cells [6]. NO is an extremely reactive gas, and by carrying an uncoupled electron, acts as a free radical. Oxidative damage, if limited, could result in apoptosis. In fact the complex phenomenon of cell suicide is characterized biochemically by an imbalance in the intracellular redox state. The pro-apoptotic activity of NO is mediated by the transcription of the guardian of the genome, the tumour suppressor protein p53. Indeed, the Amadori products of glycated albumin induce p53 expression. Although these are in vitro results, one can speculate that a similar mechanism operates in vivo. A progressive loss of mesothelial cells is observed in long-term PD patients, which results in a non-physiological contact of the peritoneal matrix and vessel with injurious molecules, such as glucose or AGEs, that is a major trigger of peritoneal fibrosis. Moreover, glucose-degradation products (GDPs) which have an oxidant activity, exert an apoptotic effect on mesothelial cells in vitro [7]. This effect differs significantly for different concentrations of GDPs as described by Nilsson-Thorell et al. [8], if traditional, or three-compartment bag PD solutions are used, suggesting a better biocompatibility of this last.

Armato: In preliminary experiments in which human mesothelial cells from healthy or uraemic subjects were cultured in vitro and exposed to conditions similar to those of CAPD, we did not observe significant degrees of apoptosis induction in comparison to untreated parallel controls; instead, we only evidenced a slight degree of necrosis and cytotoxicity. These findings are well in keeping with a positive TUNEL test in a fraction of mesothelial cells exposed to dialytic fluids [9]. Yet although the TUNEL test signals DNA damage, it is no longer considered to be a specific indicator of apoptotic cell death, as it gives positive results even under conditions of frank cell necrosis. It is true that under particularly intense apoptogenic stimuli, the induction and execution of apoptosis might be rapid, requiring only a few hours and this might make cells dying by apoptosis difficult to detect under the microscope. However, it must be stressed here that when apoptosis has taken place as rapidly as it might, apoptotic bodies can still be easily detected by an experienced microscopist, both inside the cytoplasm of the surviving cells and floating in the medium, and they are also detectable by flow cytometric analysis of the corresponding cell DNA samples. So other, more specific tests for apoptosis than the TUNEL one, such as analysis of cellular DNA ploidy and caspase activity, should be used to obtain a definite answer to this question.

Amore: Even if we do not find apoptotic cells in peritoneum specimens, I believe that apoptotic death has to be considered when there is a progressive loss of cells without signs of inflammation. In fact, the release of lysosomal enzymes during necrosis is always associated with neutrophil infiltration, a condition not described in peritoneal sclerosis of PD patients.

Sterzel: I’d like to add another aspect to the question of chronic changes of the peritoneal membrane in CAPD due to high glucose. It’s obvious that in chronic diabetes, the GBM changes. However, one has to be careful with analogies between the glomerulus and the peritoneal membrane. We shouldn’t ignore the mechanics. In the glomerulus, the pressure is 50 mmHg; in the peritoneum we are dealing with a very low-pressure system. The relevant question is whether a diabetic person without CAPD has peritoneal membrane changes like those found in a non-diabetic CAPD patient. The relationship to the GBM without considering pressure is misleading.

La Greca: I would like to ask Dr Coles if there are biopsies taken from diabetic patients before starting PD, and what their histological picture looks like.

Coles: As far as I know, nobody has ever described the peritoneum as thickened, in terms of having a lot of interstitial fibrosis, in diabetics before they start dialysis, and we know that functionally they do much the same for quite a number of years. This would suggest that diabetics does not start off with a much

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1 Amadori products/compounds are defined as amino-acid derivatives formed via a condensation reaction (nucleophilic addition with subsequent rearrangement) between the carbonyl group of reducing carbohydrates (e.g. glucose, fructose, or other glucose-derived carbonyl compounds, etc.) and an amino group of amino acids, peptides, or proteins. Amadori products are reactive, labile intermediates of the initial stage of the so-called Maillard reaction, which finally results in AGEs.
worse functional membrane to begin with, and would be against diabetes alone being the cause of the problems.

La Greca: Generally speaking, there is a great current interest in gene therapy. Is there any chance of applying it to PD? Which molecules or cells might be the targets of peritoneum gene therapy? Aquaporins, perlecans, transforming growth factor-β (TGF/β), vascular endothelial growth factor (VEGF) and other angiogenic factors (angiopoietin I and 2)? Cytokines (interleukins, tumour necrosis factor alpha (TNFα))? Surfactants? Which cells; endothelium, mesothelium or interstitial fibroblasts?

Esposito: It’s quite difficult to answer this question. Numerous factors are probably involved. In order to make gene therapy successful we must have a specific target. However, we could approach the problem differently. The endothelium is surely the first target of AGEs [11]. In fact, endothelial cells express the receptor for AGEs, called RAGE, and the binding of AGE to its receptor modulates endothelial functions [12]. The blockade of AGE-RAGE was shown to prevent vascular changes in an experimental model of diabetic angiopathy. Stern’s group (Wautier et al.) [13] recently produced a truncated soluble form of RAGE, termed sRAGE, using a baculovirus expression system. This soluble receptor competes with the cell surface receptor for binding to AGEs, and is suitable for long-term experiments. It would be great if we could inject sRAGE into the peritoneum of our patients and block the effects of AGEs; a study like this would enable us to understand better the real role of AGEs in the pathogenesis of peritoneal fibrosis, and hopefully to find a way to delay this process.

Feriani: If something has to be done, I as a clinician would prefer an intervention on aquaporin, because ultrafiltration is the most important problem in PD patients.

Mason: The list of molecules suggested by Professor La Greca is long and impressive. However, I don’t think that any one of them is an appropriate target for gene therapy at the moment because we don’t know each one’s precise role in peritoneal fibrosis. Secondly, I think it’s very unlikely that peritoneal fibrosis and loss of function will be attributed to any single factor. So if you were thinking about any sort of gene therapy, you would probably have to think of multiple approaches, and that is very difficult, particularly with gene therapy. So although these are all worthy subjects for investigation, I think it is too early for gene therapy.

La Greca: Notwithstanding these negative comments, I wish to force the discussion in this direction. Which systems might be used to engineer the peritoneum? Transfected mesothelial cells, viral vectors, liposomes? Should we use the systemic or the peritoneal route to introduce these factors? Are there any examples in other illnesses that might apply here?

Noonan: Vector systems are one of the key problems in gene therapy and I think that this is what you really mean. We don’t even know what target cell types we are talking about either, and so I would be very hesitant to even attempt to make a guess as to what would be the best systems to use.

Mason: I agree with that, but I suspect that when it comes down to it, viral vectors are probably going to be the way forward for gene therapy in general, and on that basis alone, I would say that they are the most likely candidates as vectors in the future.

La Greca: What is the clinician’s viewpoint, Professor Coles?

Coles: My gut feeling is that we should start by preventing peritoneal dysfunction and fibrosis in the first place by improving the un-physiology of the dialytic fluids. I don’t know if gene therapy is proven to do anything to patients as yet. What little I know about it is that in small rodents you can apply gene therapy, or you can use transgenes in mesothelial cells and get them re-implanted [14], but it’s probably not the mesothelial cells that matter anyway. Furthermore, I understand that there is a slight risk that the cells will actually be taken up by the lymphatics, and so you won’t get just a localized effect if you use whole cells.

Sterzel: May I challenge the panel? In CAPD patients, you have a unique chance to introduce a gene four times a day, every single day, because you have immediate access to the patient’s peritoneum. Gene transfer seems attractive. For example, consider the problem of intraperitoneal coagulation and fibrin formation causing adhesions and malfunction. Of course, there is heparin. But wouldn’t it be timely to ask whether there are ways to increase fibrinolysis by introducing genes into mesothelial cells in order to promote their fibrinolytic activity? I think we should stick with the issue of local gene transfer in CAPD patients a bit longer.

Mason: I hate to sound negative, but I think it was Sir Peter Medawar who said, ‘Science is the art of the possible’. We may have a unique opportunity to introduce genes into patients four times a day, but even introducing genes into cells in vitro and getting them to express efficiently is frequently unsuccessful.

La Greca: Okay, it’s a dream. There are some reports concerning the auto-transplantation of peritoneal cells in order to renew the ultrafiltration capacity of the peritoneal membrane. Very fascinating. Is this a reasonable possibility?

Armato: That might be a possibility, although its degree of practical feasibility remains to be established. Here we should recall that peritoneal mesothelial cells include a stem-cell compartment [15], that is essential for cell replacement. Being able to proliferate, these stem cells regenerate the mesothelial layer after acute cell damage and/or loss. Of course, what is usually done during CAPD is the injection of a cytotoxic solution into the peritoneal cavity, which will end up damaging mesothelial cells, including those pertaining to the stem-cell compartment. This does not involve the actual killing of many cells, but it determines DNA damage and changes the gene expression pattern of most of the mesothelial cells, stem cells included. As a consequence, the p53 gene is activated, its protein product switches on the p53-slave genes, including p21, and the proliferation of the mesothelial stem cells is...
blocked, probably even for long intervals. Since few peritoneal stem cells are likely to remain intact after a CAPD session, and these are only able to proliferate slowly, the process of regeneration or of normal mesothelial cell replacement remains impaired for quite a while. Therefore, by repeating the CAPD procedure so many times, progressive attrition of the mesothelial cells will be unavoidable due to cytotoxicity coupled with the protracted decrease in the size of their growth fraction. This will eventually lead to portions of the peritoneal surface remaining devoid of their mesothelial lining. Moreover, treatment with a PD solution decreases the adhesion of the mesothelial cells to their basal lamina by changing the expression of some of the integrins. Hence, cell loss during CAPD via decreased adhesion to the substrate, and impaired cell regeneration via stem-cell DNA damage will conspire to reduce progressively the actual surface of the mesothelial cell lining of the peritoneum, and thereby the clinical effectiveness of CAPD.

**Schleicher:** If the stem cells proliferate very slowly, wouldn’t it be possible to add recombinant growth factors, i.e. colony stimulating factors or something like that?

**Armato:** Actually, this is easily feasible. Among the various growth factors, recombinant human epidermal growth factor (rhEGF) could be a good candidate, since Pronk et al. [16], showed that the administration of EGF (and hydrocortisone) can enhance the proliferative activity of human mesothelial cells cultured in vitro even in the presence of low serum concentrations. This is in keeping with the demonstration that normal human mesothelial cells cultured in vitro do indeed express the EGF receptor (EGF-R) [17]. In addition, it was recently reported that EGF facilitates the healing of the peritoneal membrane after acute injury by augmenting both mesothelial cell adhesion to the basal lamina and migration into the wound area [18]. However, I must also add that Mutsaers et al. [19] found that EGF was not an effective mitogen for mesothelial cells in vivo, which might well be true if the peritoneal lining is intact when exposed to EGF, and has not undergone previous acute injury.

**La Greca:** Let’s now address the problem of the un-physiology of PD solutions and the relative role of AGEs. In a paper published in 1994 in *Nephrology Dialysis Transplantation* (Ritz et al. [20]) Dr Deppisch predicted that the nephrological community would be busy for the next 10 years trying to answer a number of questions on AGEs (Table 1). At which point are we now? Can we also define a toxicity of glucose itself?

**Deppisch:** Without doubt the scientific community has made good progress in this field over the past 5 years. Yes, AGE peptides are pathogenic; it is worthwhile to remove them, or prevent their uptake and formation. AGE peptides specifically interact with RAGEs. AGE peptides certainly contribute at different levels to the sequelae of connective and vascular tissue rearrangement. The important point we have understood is that we have to work with more specific and better defined AGE products instead of using just group reactivity, and this will certainly help to identify key pathophysiological routes. We still lack sufficient knowledge concerning the relative importance of different AGE species and their precursors like carbonyl compounds, such as early or late products, i.e. Amadori intermediates, pentosidine, pyrraline, carboxymethyllysine (CML), etc. We cannot continue to work with a ‘soup’ prepared in vitro by ageing albumin for 10 or 30 days, and then perform biological assays with such a poorly defined mixture of AGE products. By working with specific precursors or AGE products, I believe that in the next 5 years we will be able to make better analogies between the situation of the peritoneal tissue and diabetic complications in order to understand the molecular role of carbonyls and AGEs in the development of fibrosis, neangiogenesis, and vascular damage. It will probably be possible to combine the fields of diabetes and peritoneal tissue research to reach good and useful conclusions.

**Mason:** I just want to comment about the toxicity of glucose. High glucose is not just toxic because it leads to the formation of AGE products or to metabolic products, which have downstream effects, but because of its own effects; within minutes of mesangial cells exposure to 30 mmol/l glucose, the cell kinase signalling pathways, i.e. the classic MAPkinase p42/44 and the p38 pathways, are activated for at least 24 h [21]. The importance of this is that these pathways are capable of phosphorylating transcription factors, for example the CREB transcription factor, which in turn are capable of binding to sites within the promoters of genes encoding various matrix molecules, like fibronectin, decorin, and so on. So exposing a mesangial cell to a very high glucose concentration, and 30 mmol/l is not as high as some of the ones used in CAPD, leads to downstream effects on the promoters of matrix encoding genes. If you keep doing this to mesothelial cells in CAPD, you’re going to promote a fibrotic situation.

**La Greca:** Which AGEs appear or might appear during PD? Do the necessary conditions exist (i.e. time) for them to come into effect and exert a

### Table 1. Questions on AGEs (Taken from [20].)

- Are circulating AGE peptides pathogenic?
- Is it worthwhile removing them?
- Do products such as FFI or pentosidine interact in vivo with monocyte receptors of uraemic patients?
- Are low-molecular-weight AGE peptides deposited in extravascular tissues?
- What types of AGE are important? FFI, pentosidine, both types, or others?
- Do they contribute to the pathogenesis of atheroma, stiffening of connective tissue, and the accelerated ageing of dialysed patients?
- If ‘yes’, can this outcome be prevented by their removal, reduced generation, and/or manipulation of their receptors?
toxic action during PD? Which AGEs are potentially to blame for causing peritoneal damage?

Deppisch: The reaction sequence starting from glucose and GDPS, which are chemically identical to carbonyl stress compounds, and finally leading to AGE products is time-, temperature- and pH-, that means environment-dependent. Therefore it is difficult to predict which specific modification, e.g. pentosidine, pyrraline, CML, imidazolone, immediate early Amadori products or many thus far unidentified other products, will dominate in the signalling processes involved in tissue restructuring. In PD fluids, the identification of GDPS allows a certain prediction of relevant pathways in the AGE reaction scheme. Due to its high concentration, 3-DG (3-deoxyglucosone) seems to be an important carbonyl stress compound in the AGE sequence. Glyoxals are also important since they are highly reactive candidates. Aldehydes are thought to be highly cytotoxic in the micromole range.

It seems to be important to keep in mind that different GDPS as well as different intermediate products in the Amadori and the Maillard pathways have different reaction kinetics. They are all highly reactive in the presence of amino groups. This means that the reaction sequence can be triggered in minutes, but it could take time (hours or days) to get the modifications along the Amadori and Maillard reactions completed. In this dynamic process, the difficulty of analysing the biologically relevant signal pathways seems to be obvious.

Schleicher: Dr Deppisch has mentioned several AGE structures that have been identified and partly measured in diabetic patients, but CAPD-treated patients are less well studied. Besides identification and quantification of the AGE products, we need to know if these products are biologically active. Up to now there is little information on the bioactivity of defined AGEs like pentosidine, pyrraline, or imidazolone. However, recent data indicate a function for CML-modified proteins. Kislinger et al. [22] have shown that CML-modified proteins bind to RAGE and activate the signalling pathway, i.e. activate NFκB. Although not studied in detail, CML modification may be a major AGE product formed under PD conditions, because it is the major AGE product formed from Amadori products under oxidative conditions.

La Greca: Is the action of those AGEs that come into effect during PD merely local, or systemic?

Deppisch: Professor La Greca brings up here a fundamental question to be addressed for a more precise definition of the pathophysiology initiated by the exogenous uptake of carbonyl stress products from PD fluids. Considering the highly reactive properties of carbonyl stress compounds, it is reasonable to assume that the cellular constituents and matrix elements of peritoneal tissue should be primarily affected. Immuno-histochemical analyses by Nakayama et al. [23] clearly showed the formation or deposition of AGE products primarily at the capillary walls and later in the interstitial connective tissue. To my knowledge, to date there is no data showing that the increased exogenous uptake of GDPS can also involve peripheral blood; but if we consider the large amount of fluids exchanged in PD for an effective treatment, I would not exclude a systemic effect. This question is currently the centre of our interest and laboratory work.

Feriani: Personally, I am not so sure of that. Indeed, despite continuous glucose loading in PD, the serum level of pentosidine is significantly lower in PD patients compared to HD patients. PD dialysate contains a high proportion of high-molecular-weight AGE proteins, whose concentration is similar to that of serum [24]. Concentrations of AGEs are significantly higher in dialysate than in serum when both concentrations are standardized with their protein concentrations. Furthermore, peritoneal clearance of the albumin-bound form of pentosidine exceeds the clearance of free albumin [25]. These findings suggest a ‘wash-out’ effect of PD. It could be hypothesized that the glucose and carbonyl compound load during PD produces an intraperitoneal formation of AGEs, and a consequent equilibrium between formation and removal. Thus, the effect seems to be local rather than systemic. This view is further supported by the finding that, while the pentosidine content of skin collagen is similar in patients treated with PD and HD, the pentosidine content of peritoneal tissue is significantly higher in patients on PD than in patients on HD [26].

Amore: We are talking about AGEs, but we must not forget about the Amadori products that also react, and induce the same effects in mesangial and mesothelial cells that are induced by AGEs. So, this is one more reason why we have to think about another possible cause of systemic effects, in addition to glucose and AGEs.

Schleicher: This is an important notion. Only a very few groups are investigating the effect of Amadori compounds, but it appears that in addition to RAGE, a receptor for Amadori compounds is expressed on cells, and when stimulated its activation leads to cytokine expression.

La Greca: Do AGEs have a role in PD patients with peritonitis? Do they have a role in infections?

Coles: There is no data to answer this question. Current fluids do impair host defence temporarily in vivo, but this is due to the low pH and the lactate [27]. Furthermore, the clinical significance is unknown. Since mesothelial loss occurs during peritonitis, it is then possible that AGEs could more easily penetrate the interstitium and affect fibroblasts and endothelial cells, as well as the collagen already present. This is at present pure speculation.

Deppisch: We performed a series of investigations on the effects of carbonyl stress compounds on different cell types in vitro. For example, the presence of GDP (= carbonyl stress) induces cytotoxicity in fibroblast cultures, and among others, it reduces the normal cytokine release pattern generated by mononuclear cells [28]. Other groups have also shown that carbonyl stress compounds suppress the normal function of granulocytes in the host defence [29,30]. Summing up this issue, I believe that besides the general cytotoxic effects...
of GDPs, they most probably also have a negative impact on the different cell types involved in physiological defence mechanisms during peritonitis. To date, it has not been possible to get clinical data on this question, but with the new GDP-free fluids, for the first time we have the possibility of performing prospective studies in this direction.

La Greca: We have learned a lot today on glucose-related toxicity. Is there any strategy to prevent it? Dr Schleicher! What’s the biochemist’s point of view?

Schleicher: We learned that glucose is toxic by itself, and also because it is degraded during the sterilization procedure. Therefore, the first point to be addressed would be to substitute glucose with another less reactive sugar carbohydrate. For example, sorbitol, as some have proposed. It was also suggested that fructose could be a substitute for glucose. I would say that fructose is even worse than glucose because it’s even more reactive, and forms AGEs much faster than glucose.

The next point of intervention would be to block the reaction of glucose with proteins forming AGEs in the extracellular fluid. We are still outside the cells and therefore we have the possibility to interfere with AGE formation. Preliminary studies in animals found that the addition of aminoguanidine, an inhibitor of AGE formation, to the PD fluid had a positive effect. However, oral aminoguanidine caused severe side-effects in diabetic patients during clinical testing. Since low-molecular-weight drugs are readily taken up from PD fluid, it may not be advisable to add this drug. I am not aware of new molecules acting on AGE formation that are ready to be tested in the clinic. We may try to block the biological effects of the formed AGE products, or of glucose itself, i.e. gene activation which causes cytokine formation and extracellular matrix deposition. For instance, AGE products bind to RAGE, thereby activating the transcription factor NFkB that leads to cytokine formation. So, any agents working on RAGE, or on the downstream signalling cascade up to NFkB activation could be useful in theory.

Because activation of this signal transduction pathway can be due to an intracellular oxidative stress, it may be inhibited by reagents which have a reactive sulphur group, like glutathione, which, however, is not very effective since it is not easily taken up by the cell; or alpha-liponic acid, which has been shown to block the activation of the NFkB system induced by AGEs in diabetic patients [31]; or N-acetylcysteine; or reductive vitamins like vitamin E rather than vitamin C, because vitamin C may also form a reactive carbonyl compound, and could therefore increase AGE formation.

La Greca: And what’s the biotechnologist’s point of view?

Deppisch: We know that heat sterilization, long-term storage, and a solution above pH 5 are the major factors involved in glucose degradation, and the formation of reactive carbonyls, finally leading to toxicity. From today’s perspective, multi-compartment technology protects glucose from degradation during sterilization and long storage periods through the separation of highly concentrated glucose at a pH of about 3 from the other buffer and ion components. The use of ultrafiltration methods for sterilization at low temperature is not possible at present due to pharmacopeial requirements. Additionally, sterilization does not solve the problem of glucose degradation during long storage periods. At present, the multi-compartment technology with highly concentrated glucose at low pH provides PD fluids with carbonyl concentrations below 30 μmol/l at the time of fluid installation. This is a key criterion for a long-term treatment modality, applying several thousand litres of fluids per year.

La Greca: Are there any possibilities that have not yet been considered? The use of glycosaminoglycans (GAGs)? Which ones? Chondroitin sulphate or low-molecular-weight heparins and/or dermatan sulphate? Other drugs? Which ones?

Deppisch: Generally speaking, this is a challenging question which needs to be answered in a stepwise fashion: first we need to prevent the basic cytotoxicity mainly induced by glucose degradation due to unfavourable conditions in manufacturing related to pH, temperature, and storage. Second, we need to elaborate whether pure glucose or non-GDP contaminated fluids are able to induce signal pathways involved in tissue restructuring. And third, we have to evaluate the alternatives to glucose and other additive substances, that can be chronically applied in large quantities for different purposes in PD therapy. The list of reasonable candidates gets longer and longer: GAGs, vitamins, nutritional supplements, anti-oxidants, and many others can be envisaged today, but to my knowledge, a really hot and promising candidate is not presently under clinical investigation, nor available in the near future for a broad clinical application in routine PD therapy.

Schleicher: The idea of using GAGs deserves more attention. The Padova group [32–34] first showed that GAGs, like low-molecular-weight, low-anticoagulant heparin fractions or dermatan sulphate, could prevent diabetic nephropathy in experimental animals, in particular GBM thickening and mesangial expansion, by inhibition of collagen synthesis. Studies on the molecular mechanisms revealed that GAGs exert their beneficial action by inhibiting TGF-β1 overexpression, which is the key mediator in the development of sclerotic and fibrotic diseases. Therefore, the addition of GAGs, which are already used for other indications, to the PD fluid may be considered.

La Greca: What about chelating agents?

Schleicher: To my knowledge and from our own experiments, chelating agents are effective in blocking oxygen-derived AGE formation. Since trace elements like iron are needed to catalyse the oxygen-radical-mediated reaction blocking this activity, the chelation of the trace metals works beautifully in vitro. However, we always have traces of iron or copper or other metals in vivo, and it is not possible to chelate all these minerals also because we need them for life.
Perhaps the panel could comment on why we need glucose to make osmotic power in peritoneal solutions? Deppisch: Why is glucose important and necessary for years to come? It is not expensive, and it is available in large quantities. But perhaps more relevant to its use as an osmotic agent in PD is the knowledge of its metabolic pathways. Many potential alternative substances were investigated in the past, but they didn’t work or at least didn’t work successfully in clinical practice. Of course, from today’s perspective many gradient solutions are theoretically possible. However, it could take 10 years to get the full set of pharmacological, metabolic, toxicological, immunological, and finally clinical data on other substances that need to be intraperitoneally loaded in huge amounts into the organism. Also, a complete industrial process additionally needs to be developed and safely operated. It will certainly be a long journey to reach routine clinical use. PD is not just a small pill per day, but a home care therapy applying up to 10 or 25 litres of therapeutic fluids per day.

La Greca: This is the point of view of the clinical nephrologist. We have been looking at the last 10 years, and we are still looking for a substitute for glucose.

At the end of this brainstorming session between clinical nephrologists and basic science investigators, different interesting opinions emerged that I certainly believe will be developed over the next few years. More specifically, there have been a huge number of studies on the effect of different conditions on the mesothelium. However, it is astonishing how the mesothelium is greatly ‘absent’ from the scenario of chronic PD, since it seems to be deranged very early. Is it possible that this epithelium only has a ‘protective function’ that could give the peritoneum a non-specific resistance to infections? Can we exclude that the mesothelium also has ‘trophic’ effects on the peritoneal interstitium? This is still completely unknown but were this the case, its role would certainly be much more important than thought, and perhaps decisive in conditioning the survival of PD in the single patient.

Professor Armato’s data reminded us of the existence in the peritoneum of staminal totipotential cells, and I found it very stimulating. What is the precise physiopathological role of these cells in chronic PD? Is it possible to ‘manipulate’ or stimulate them to favour a more physiological remodelling of the peritoneal membrane, and therefore possibly keep peritoneal membrane function as normal as possible? Can we use these cells for autotransplantation to reconstitute an effective anatomic barrier in patients with severe peritoneal dysfunction? I believe that these aspects have been neglected, and that they merit major attention.

The panel was categorically against the possibility, at least in the short-term, of gene therapy, primarily because it is not easy to identify a target on which to act. Nevertheless, I believe that we already have a very important target for the long-term survival of this dialytic method in uraemic patients. In functional terms, this target is the loss of ultrafiltration capacity, and if it is true that aquaporin has an important role in this function, then we have a molecular target on which to act. It is clear that our knowledge of the physiopathology of water transport mechanisms in the peritoneum, of ultrafiltration deterioration during chronic PD, and of the role of aquaporins is probably still not sufficient for this type of approach. Nevertheless, returning to Professor Sterzel’s suggestion, what is the best situation for gene therapy of a condition such as PD in which the target tissue, i.e. the peritoneum, is treated four times a day every day?

Our carbonyl stress experts thoroughly explained the complexity of the problem, and in fact could also suggest that even in this physiopathological pathway it is possible to envisage gene therapies or cell pharmacology strategies. Nevertheless, my feeling is that modifying the dialytic solutions is certainly the shortest and most suitable method at hand for improving PD. Recent examples of how PD solutions are evolving support this idea. As a matter of fact, the substitution of lactate with bicarbonate has practically solved the problem of the excessive acidity and chemical stress induced on the peritoneum by a lactate-containing PD solution. In recent years, new strategies to overcome the problem of ‘glucose toxicity’ have also been proposed: icodextrin, glycerol, amino-acid solutions, and three-compartment bag glucose solutions. The new PD solutions do not completely solve the problem of glucose toxicity, and some moreover raise different clinically relevant issues: when using icodextrin PD solutions, are the chronic high levels of maltose observed really safe? What about the cardiovascular risk conferred by glycerol solutions, and the metabolic and nutritional disturbances due to amino-acid PD solutions? I believe that overcoming the still numerous knowledge gaps regarding the mechanisms of un-physiology of dialytic solutions will enable us to make significant progress in this field.

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