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ORIGINAL ARTICLE

Biocompatibility of a new PD solution for Japan, Reguneal™, measured as in vitro proliferation of fibroblasts

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Abstract

Background The aim of this study was to investigate in vitro biocompatibility of Reguneal™, a new bicarbonate containing peritoneal dialysis fluid (PDF) for Japan, and compare it with other PDFs available in that country.

Methods We assessed basal cytotoxicity using in vitro proliferation of cultured fibroblasts, L-929, determining the quantity of living cells by the uptake of Neutral Red. Levels of ten glucose degradation products (GDPs) were measured by a validated ultrahigh-performance liquid chromatography method in combination with an ultraviolet detector. We compared inhibition of fibroblast cell growth between brands of PDF, adjusting for dextrose and GDP concentrations using random-effects mixed models.

Results The results demonstrate that cytotoxicity of Reguneal™ is comparable to a sterile-filtered control and is less cytotoxic than most of the other PDFs, most of which significantly inhibited cell growth. As a "class effect", increasing dextrose and GDP concentrations were non-significantly but positively associated with cytotoxicity. As a "brand effect", these relationships varied widely between brands, and some PDFs had significant residual effects on basal cytotoxicity through mechanisms that were unassociated with either dextrose or GDP concentration.

Conclusion Our study suggests that Reguneal™ is a biocompatible PDF. The results of our study also highlight that dextrose and GDPs are important for biocompatibility, but alone are not a complete surrogate. The results of our study need to be confirmed in other tissue culture models, and should lead to further research on determinants of biocompatibility and the effect of such PDFs on clinical outcomes.

Keywords Biocompatible · Peritoneal dialysis · Glucose degradation products

Background

At the most recent census, there are over 320,000 patients on dialysis in Japan. However, only 2.7% of these are currently being treated with peritoneal dialysis (PD), with the rest being treated with hemodialysis (HD). This proportion is much lower than the global average of approximately 12%, and is a function of a low proportion of incident dialysis patients starting PD in that country. For instance, in 2015 this proportion was only 5.6%, as compared with 9.6% in the US, 17.8% in Canada, 20% in the UK, 38% in Australia, and 56% in New Zealand. This low uptake of PD in Japan has remained virtually unchanged for 15 years, despite the acknowledged benefits of PD in providing a high level of patient satisfaction, good lifestyle flexibility, and superior facilitation around return to employment compared to HD [1].

A number of factors are responsible for this current situation. Firstly, there is a strong clinical culture of HD in Japan, which is associated with arguably the best survival in the world. As such, many practitioners in Japan do not see an unmet need with respect to clinical outcomes on dialysis,

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and will default to a prescription of HD without further consideration. A second factor is the poor profitability of PD to providers, insofar as reimbursement for PD is similar to that for HD, but more troublesome and resource intensive in Japan where the average number of PD patients per clinic is 7. A third factor is the decreasing exposure of nephrology trainees to PD, and a subsequent lack of confident PD practitioners, much less PD champions. Despite these problems, the future of PD in Japan is felt by many to be reasonable, contingent upon increased reimbursement from payers, and the development of comprehensive clinical networks to better support PD practitioners as they strive for clinical and programmatic excellence [2].

Historically, the decline of PD in Japan originally started with the so-called encapsulating peritoneal sclerosis (EPS) "epidemic" in the 1990s [3, 4]. As a result, there was widespread call to action in Japan for peritoneal protection during PD, and the early and complete adoption of biocompatible PD fluids (PDFs) in routine clinical practice. Since that time, the incidence of EPS has decreased drastically [5–7], although anxiety persists reinforcing the culture of early and planned discontinuation on PD and other special cares [8–10]. While the main threat of EPS is historical, the quest for optimally biocompatible PDFs has been an important background activity in Japan, to ensure ongoing safe and effective products for practitioners and patients.

In this paper, we examine the biocompatibility of a new PDF in Japan [11, 12], and compare this fluid to others that are commercially available in that country. The aim of this study was to compare the basal cytotoxicity of these PDFs, and to assess the relationship between measured basal cytotoxicity and the concentration of both dextrose and glucose degradation products (GDPs) within the different PDFs available in Japan. We made the assessment of basal cytotoxicity using an in vitro proliferation of cultured fibroblasts, L-929, as basic cellular mechanisms are similar in both specialized and non-specialized cell types. We made the assessment of GDPs by ultrahigh-performance liquid chromatography (UPLC).

Methods

Materials and PDF preparation

All tissue culture plastics were purchased from Nunc (ThermoFisher Scientific, Waltham, MA, USA). The following instruments were used for the measurement of GDPs. Waters Acquity UPLC binary solvent, sample and column managers, column heater and cooler (Water, Milford, MA, USA; Waters TUV Acquity detector, Tunable UV detector, Photodiode Array detector; Waters Empower Build 1154 acquisition and processing software; Mettler Toledo analytical balance (XP 205 DR/M, XP 205/M, XS 204/M) (Mettler-Toledo Pte Ltd., Singapore); Sartorius analytical balance (CP 3202 S-OCE) (Sartorius Lab Instruments GmbH & Co., Goettingen, Germany); Waters BEH Phenyl and Waters HSST3 columns; all references substances from Sigma–Aldrich (Sigma–Aldrich, St. Louis, MO, USA).

The new PDF, Reguneal™ (Baxter Japan, Tokyo, Japan), was compared with four different commercially available brands of PDFs. Since the study was intended for investigation rather than marketing, we have displayed results that do not include competitor brand names. The comparator PDFs (in random order) were the following brands: JMS Perisate NL (JMS Co., Ltd., Tokyo Japan); Terumo Midpeliq L (Terumo Co., Tokyo, Japan); Fresenius Stay-safe Balance (Fresenius Medical Care Japan, Tokyo, Japan), Baxter Dianeal-N (Baxter Japan, Tokyo, Japan). The detailed composition of the individual fluids that we tested is provided in Table 1. The commercially available PDFs had been heat-sterilized by the manufacturer.

We standardized the configuration of PDF products to ensure the comparability of PDFs from different manufacturers. First, twin-bag systems have more "mass" (i.e., plastic materials) and additional "dry-sites" compared to single bag ones, and some manufacturers (e.g., Baxter) increase the length of the sterilization cycle. This difference will differentially affect GDP formation, despite the identical PDF formulation. To avoid such confounding, we therefore used only twin-bag configurations for testing. Second, it is possible that cations facilitate glucose degradation during heat sterilization [13]. This, and the general clinical recommendation for low-calcium PDFs in evidence-based clinical practice guidelines [14–18], led us to use only low-calcium PDF configurations for testing. Finally, the volume of the PDF is a strong determinant of the exposure period in the autoclaves, and we only tested product of 2 L volume.

As a control for these commercially available PDFs, we produced a PDF-like solution in our laboratory with the following composition: 15 g (1.5%), 25 g (2.5%) or 40 g (4%) glucose, 5.4 g NaCl, 190 mg $CaCl_2$, 51 mg $MgCl_2.6H_2O$, 9 g 50% DL Lactate. This laboratory-made, PD-like solution was sterile-filtered (0.22μ m) before use.

L-929 cultured fibroblasts

A mouse fibroblast cell line L-929 was grown as a monolayer in Eagle's MEM containing non-essential amino acids (1%), supplemented with 10% fetal calf serum, 50 μg.mL gentamicin and 2 mM L-glutamine (Gibco, ThermoFisher Scientific, Waltham, MA, USA). The cultures were subcultivated twice a week using 0.1% trypsin in Ca^{2+} and Mg^{2+}-free phosphate solution. The cultures were maintained at 37 °C in humidified air with 5% CO_2. L-929 cells were taken from the subconfluent culture and seeded

Table 1 Characteristics of peritoneal dialysis fluids (PDFs) tested in this study

Product name	Baxter Dianeal-N	Baxter Reguneal™	Fresenius Stay-safe Balance	Terumo Mid-Peliq L	JMS Perisate NL
Post-mix pH	6.5–7.5	6.8–7.8	6.8–7.4	6.3–7.3	6.5–7.5
Bag material	Polypropylene	Polypropylene and polyamide	Polypropylene	Polypropylene	Polypropylene
Glucose (%), anhydrous	1.36/2.27	1.36/2.27/3.86	1.5/2.27/4.25	1.35/2.5/4	1.55/2.27
Glucose (%), as glucose monohydrate	1.5/2.5	1.5/2.5/4.25	1.67/2.5/4.74	1.49/2.75/4.41	1.71/2.50
Lactate (mEq/L)	40	10	40	40	37
Bicarbonate (mEq/L)	–	25	–	–	–
Sodium (mEq/L)	132	132	132	135	132
Ca (mEq/L)	2.5	2.5	2.5	2.5	2.3
Mg (mEq/L)	0.5	0.5	0.5	0.5	1
Osmolality(mOsm/L)	344/395	344/395/483	356/401/509	350/414/497	358/398
pH when mixed	6.5–7.5	6.8–7.8	6.8–7.4	6.3–7.3	6.5–7.5
pH in glucose chamber	3.5–4.5	3.2–3.8	2.8–3.2	135: 5.2–6.2 250: 5.0–6.0 400: 4.7–5.7	3.0–4.0

in a 96 well tissue culture plate at a low density of 2100 cell/cm^3 and incubated for 24 h. The growth medium was then removed and 200 µL of each test solution and control was added to eight parallel wells.

Exposure to PDFs and assessment of cell viability

The five PDFs were assayed using 2 bags for each PDF, and 2 assays per bag. 1.5 and 2.5% PDFs were mixed with 1 + 1 Eagle's MEM (MEM; Gibco, ThermoFisher Scientific, Waltham, MA, USA) plus 10% fetal calf serum (HyClone FetalClone, GE Healthcare Life Sciences, South Logan, UT, USA), > 4% PDFs were first diluted 4 + 1 with distilled water before mixing 1 + 1 with MEM plus 10% serum. The proliferation assay was performed as previously described [19], with the exception that the quantity of living cells was determined by the uptake of Neutral Red; the amount of Neutral Red taken up by the cells is proportional to the quantity of living cells. After 72 h incubation, during exponential growth, 0.3% Neutral Red Solution was added in an amount equal to 10% of the culture medium volume, and incubated for another 2 h. At the end of the incubation period, the medium was removed and the cells rinsed with Neutral Red assay fixative and solubilized in a volume of solution equal to the original volume of culture medium. The photometric absorbance was measured at 540 nm with 690 nm as a reference wavelength using a Thermo Multiskan EX photometer (ThermoFisher Scientific, Waltham, MA, USA). Inhibition of cell growth was calculated as the difference between control wells and test well and determined on 5–12 occasions, and expressed as a percentage (% ICG).

Measurement of glucose degradation product levels in PDFs

Levels of ten GDPs were measured at Baxter Research and Development (Europe) laboratory facilities in Braine l'Alleud, Belgium; acetaldehyde formaldehyde, 3-deoxyglucosone (3-DG), glyoxal (GO), methylglyoxal (MGO), 5-hydroxymethylfurfural (5-HMF), furfuraldehyde, glucosone, 3-deoxygalactosone (3-DGal), 3,4-dideoxyglucosone-3-ene (3,4-DGE). We developed and validated three UPLC methods to be used in combination with an ultraviolet detector. Method 1 was developed for furfuraldehyde and 5-HMF, method 2 for 3-DG, GO and MFO, and method 3 for acetaldehyde and formaldehyde. Full methods for preparation of solutions, standard solutions and samples, UPLC-UV analysis, and method validation are included in Appendix One, available as on line supplementary material.

Statistical analysis

Descriptive results are tabulated and illustrated using mean ± standard deviation. We estimated the independent effect PDF brand upon % ICG using two mixed models, which allowed us to model % dextrose as a continuous variable and compare the effects of different brands upon % ICG at each level of % dextrose and each level of GDP concentration (PPM).

In the primary analysis, the first mixed model specified the relationship between dextrose concentration and % ICG as a random effect, allowing both the intercept and slope of the relationship to vary for each brand of PDF. The resulting estimates can be interpreted informally as follows: the average difference in % ICG for each brand of PDF, relative

to the control PDF-like solution, generalizable within the range of observed dextrose concentrations.

In the secondary analysis, the model specified the relationship between GDP concentration and % ICG as a random effect, allowing both the intercept and slope of the relationship to vary for each brand of PDF. In this model, dextrose concentration was specified as a fixed effect. The resulting estimates can be interpreted informally as follows: the average difference in % ICG for each brand of PDF, relative to the PDF-like solution, accounting for differences in dextrose concentration, and generalizable at each point within the range of observed GDP concentration. GDP concentration was modelled as a single concentration (PPM) in all 10 GDPs.

Analyses were performed using the mixed procedure in Stata Intercooled 14.2 (Statacorp, College Station, TX, USA).

Results

Figure 1 shows the time course of proliferation following exposure of L-929 cells to Reguneal™ and control PDF-like solution. Figure 2 illustrates the % ICG for all of the PDFs, in categories according to brand and dextrose concentration. Figure 3 shows the GDP concentrations for all of the PDFs, also these categories.

As a "class effect" (i.e., assessing all PDFs together), there was a non-significant (independent) relationship between % ICG and concentration of dextrose. As shown in Figure S1 (available as online supplementary material), there was a trend towards an overall increase of 1.07 (95% confidence interval −0.73–2.88) in % ICG per one percent increase in dextrose concentration, after adjusting for the effect of GDP concentration. Similarly, there was a non-significant (independent) relationship between % ICG and concentration of GDPs. As shown in Figure S2 (available as online supplementary material), there was a trend towards an overall increase of 0.58 (95% confidence interval −0.06–1.23) in % ICG per one PPM increase in GDPs, after adjustment for the effect of dextrose concentration.

As a "brand effect" (i.e., assessing individual brands of PDF separately), the effect of dextrose and GDP concentrations on % ICG were often variable between brands, as illustrated in Fig. 4. The upper panel shows these relationship for dextrose, and the lower one for GDPs. This figure suggests that the brand of PDF modifies the basal cytotoxicity that results from dextrose and GDPs.

Table 2 shows statistical estimates from the primary analysis for "brand effect", where we only adjusted for dextrose concentrations. There was no difference in cell growth between Reguneal™ and the control PDF-like solution, with confidence intervals that cross zero. In contrast, all of the other commercial available PDFs in Japan significantly inhibited cell growth.

Table 3 shows statistical estimates from the secondary analysis for "brand effect", where we adjusted for concentrations of both dextrose and GDPs. It can be seen

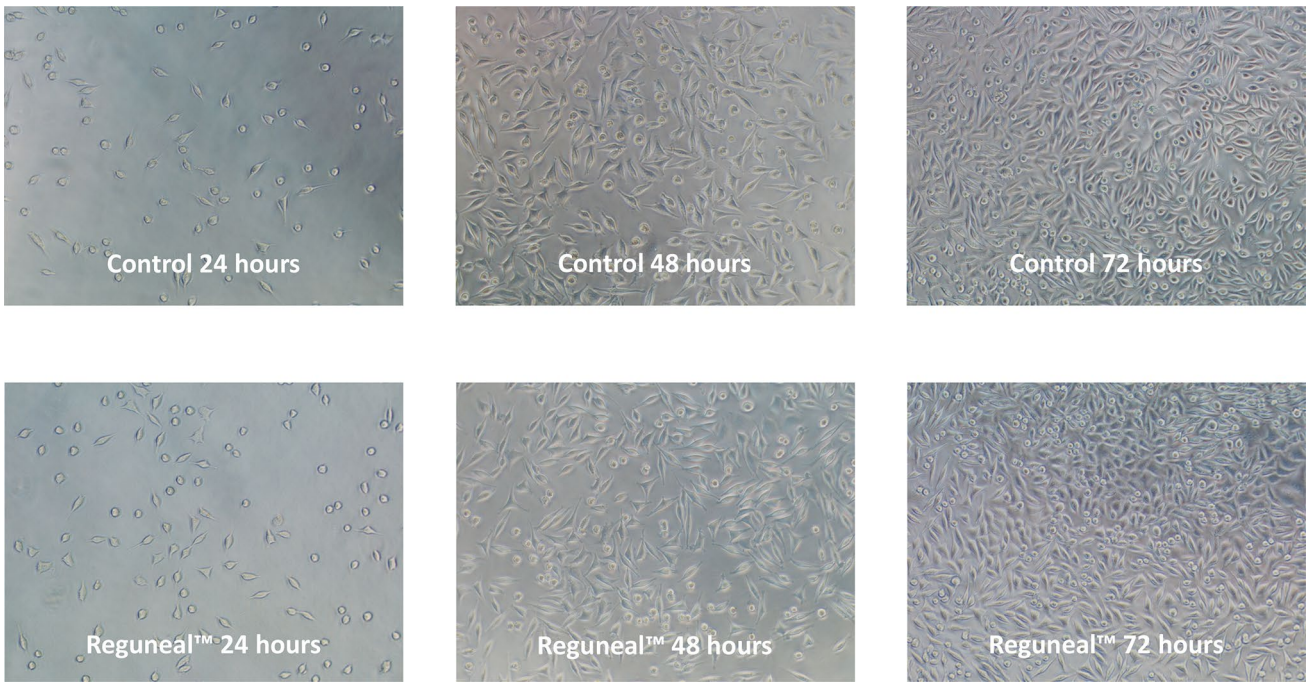

Fig. 1 Time course of proliferation following exposure of L-929 cells to Reguneal™ and sterile-filtered, control PDF-like solution

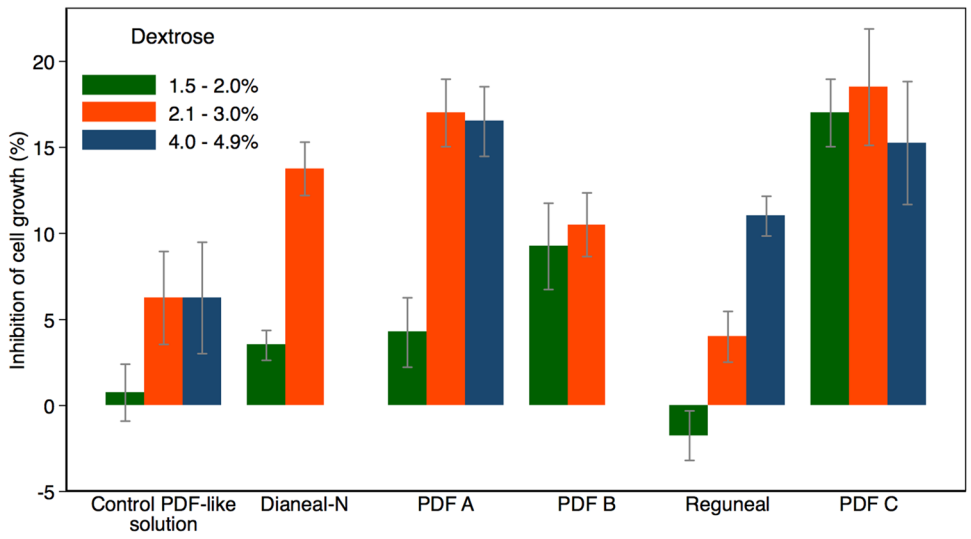

Fig. 2 Means (bar) and standard deviations (whiskers) for the inhibition of cell growth (% ICG) relative to control, by brand of peritoneal dialysis fluid (PDF), and by percentage of anhydrous dextrose

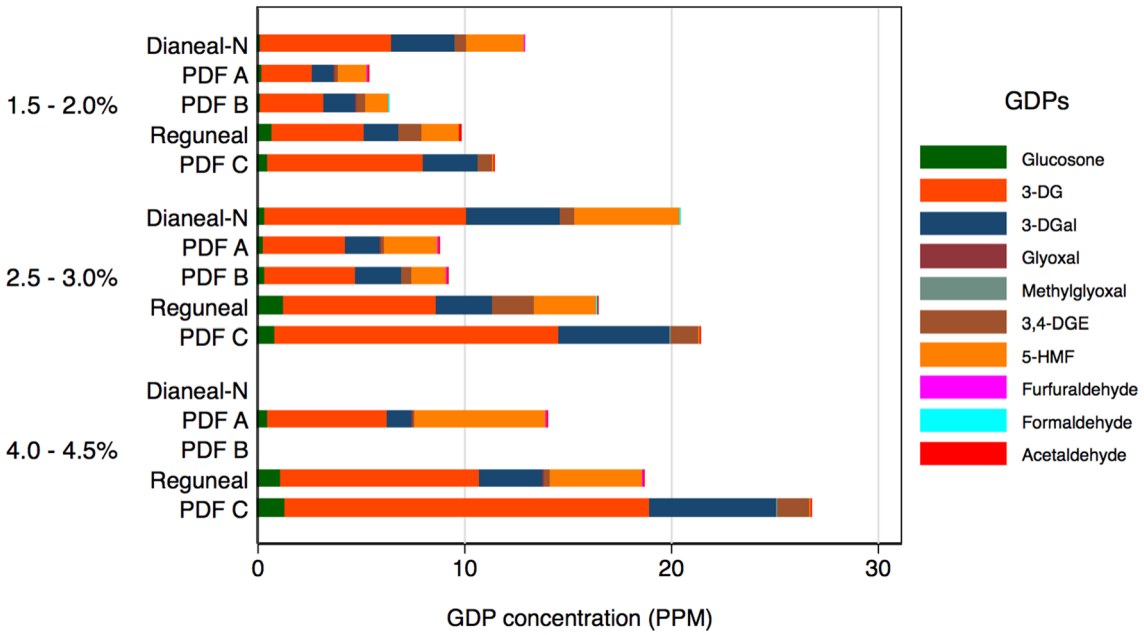

Fig. 3 Mean glucose degradation product (GDP) concentration (PPM), by brand of peritoneal dialysis fluid (PDF), and by percentage of anhydrous dextrose

that there are significant differences between the PDFs even after making this adjustment—i.e., there are cytotoxic properties of the PDFs that are not accounted for by dextrose and GDPs. Reguneal™ inhibited cell growth less than the control PDF-like solution. Of note, this was also the case for Dianeal-N in this analysis, indicating that the inhibition of cell growth that we identified in the primary analysis can be accounted for by the effect of GDPs. In contrast, all of the other commercial available PDFs in Japan were either no different or still more cytotoxic than the control solution, even after adjusting for dextrose and GDP concentrations. In particular, the inhibition of cell growth with PDF C clearly occurred through mechanisms that were unassociated with these factors.

As a sensitivity analysis, we modeled GDP concentration as the aggregate of only the GDPs that are the strongest candidates for cytotoxicity, namely, 3,4-DGE and formaldehyde [20, 21]. This did not meaningfully change the estimates or results above (analyses not shown).

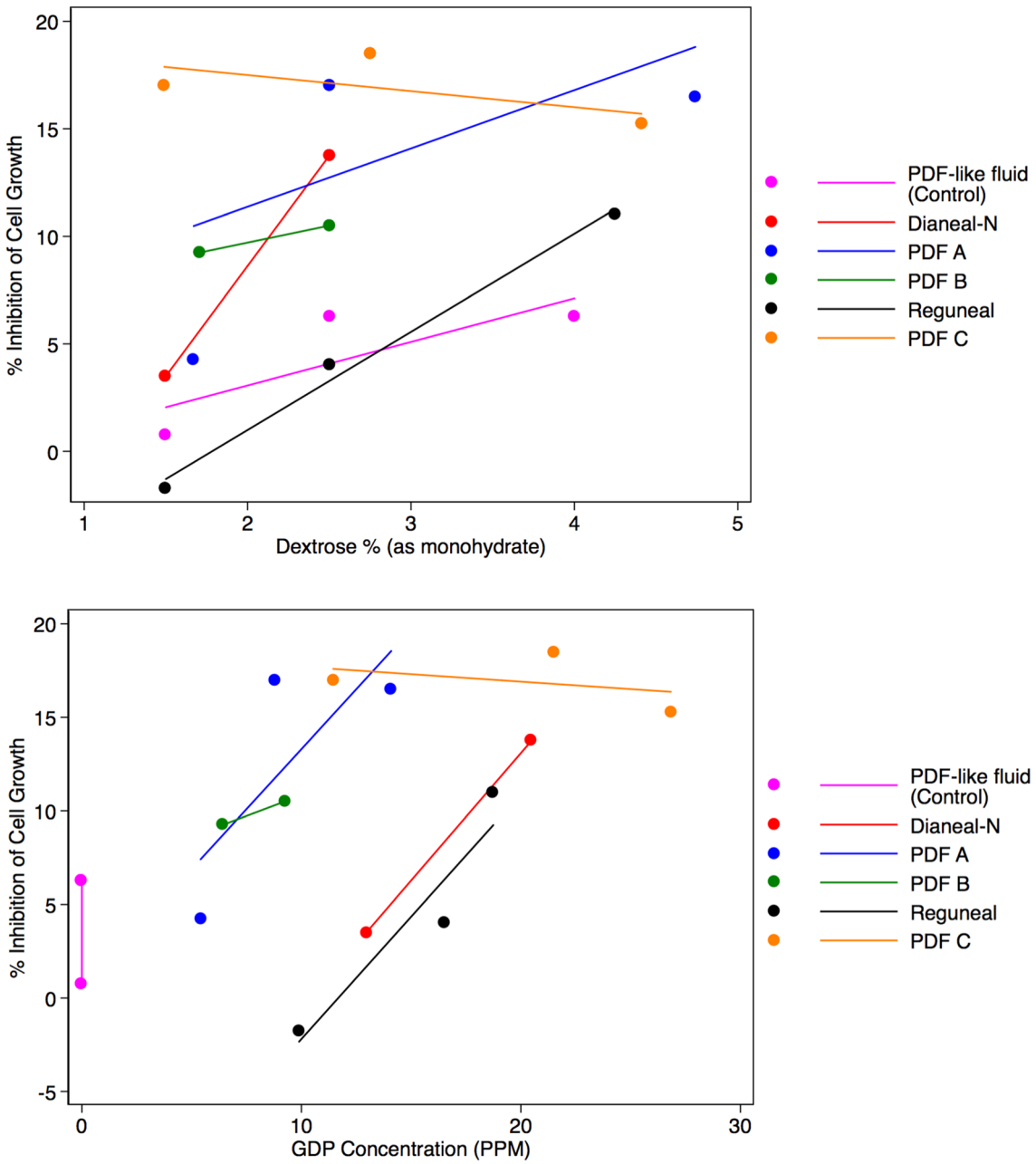

Fig. 4 Relationship between the inhibition of cell growth (% ICG) and dextrose concentration (upper panel), and between % ICG and glucose degradation product (GDP) concentration (lower panel), by brand of peritoneal dialysis fluid (PDF)

Table 2 Estimates for the marginal inhibition of cell growth (% ICG) for each brand of PDF, relative to the control PDF-like solution, after adjustment for dextrose concentration

	Difference in % ICG between the PDF and the control PDF-like solution	Lower 95% CI	Upper 95% CI	*P* value
Dianeal-N	6.87	2.48	11.25	0.002
PDF A	7.50	3.77	11.23	< 0.0005
PDF B	6.88	2.60	11.17	0.002
Reguneal™	− 0.83	− 4.56	2.90	0.662
PDF C	13.06	9.32	16.80	< 0.0005

Table 3 Estimates for the marginal inhibition of cell growth (% ICG) for each brand of PDF, relative to the control PDF-like solution, after adjustment for dextrose and GDP concentration

	Difference in % ICG between the PDF and the control PDF-like solution	Lower 95% CI	Upper 95% CI	*P* value
Dianeal-N	− 11.82	− 22.91	− 7.32	0.037
PDF A	4.71	− 6.89	7.84	0.9
PDF B	2.30	− 6.50	11.09	0.609
Reguneal™	− 13.81	− 24.03	− 3.60	0.008
PDF C	16.54	7.66	25.43	< 0.005

Discussion

The aim of this study was to investigate the biocompatibility of Reguneal™, a new PDF for Japanese with end stage kidney disease on PD [22]. There are two important characteristics of the Reguneal™ formulation; first, the reduced GDP formation during manufacturing; second, the bicarbonate-based buffer with reduced lactate, resulting in a neutral pH, aiming for physiological serum bicarbonate concentrations and pH in Japanese populations.

GDPs develop from glucose during heat sterilization (in which PDFs are heated to ~ 121 °C) and are a major cause of the cytotoxicity associated with conventional PDFs [23–27]. Focusing on 3,4-DGE, the lowest concentration of this GDP will occur during heat sterilization of a glucose containing solution at a pH of between 2 and 3 [28–30]. In turn, this is enabled by the two-chamber bag system that allows separate storage of glucose at a low pH [31, 32]. With Reguneal™, the Japanese-specific polypropylene plastic container enables the pH in the glucose chamber to be maintained between 3.2 and 3.8, thereby reducing GDP formation and improving biocompatibility.

From *in vivo* experiments, it is known that the pH of PDFs is neutralized within a few minutes [33]. During this short window, however, the low pH that is inherent in conventional, one compartment, lactate-based PDFs may adversely affect mesothelial cells, by the lowering of intracellular pH and impairment of metabolism due to changed redox potentials [34, 35]. Bicarbonate is generally advocated instead of lactate as a naturally occurring buffer that enables a physiological pH of 7.4 in solution. Of note, the wholesale replacement of lactate by an equivalent amount of bicarbonate results in a solution pCO_2 of 200–300 mmHg, as predicted by the Henderson–Hasselbalch equation. In turn, this can lead to abdominal discomfort [36], lowering of intracellular pH, and impairment of cellular functioning: in vitro experiments demonstrate that migration and function of polymorphonuclear cells is affected by the base balance, with optimal performance observed in the setting of a bicarbonate and reduced lactate formulation, compared to bicarbonate alone [35, 37–41]. With Reguneal™, the buffer formulation is the bicarbonate-based buffer with reduced lactate, thereby resulting in a neutral pH without paradoxical intracellular acidification, and improved biocompability.

Our study suggests that Reguneal™ is a biocompatible PDF. Our findings are supported by another recent study, which observed a reduction in markers of peritoneal membrane failure in effluent dialysate after transition from another PDF to Reguneal™ [12]. On the basis of these results, the enhanced biocompatibility of Reguneal™ might ameliorate PDF-induced peritoneal membrane remodeling, and lessen the frequency and severity of peritoneal membrane failure. There is some support for this possibility in a recent landmark study from Japan [5]. This study showed that the decline in EPS was most strongly predicted by the use of more biocompatible PDFs, rather any practice around early and planned discontinuation [42, 43]. Of course, more studies are needed before this hypothesis can be accepted.

Our study has several important learning points around biocompatibility. The first is that for biocompatible PDFs, basal cytotoxicity is related as much to dextrose and other unknown mechanisms as it is to GDPs. In our study, PDFs that we tested were all relatively biocompatible, with relatively low % ICG. For comparison, % ICG ranges from 50 to 75% for conventional PDFs. It is therefore unsurprising that GDPs did not exert an exceptionally strong effect upon basal cytotoxicity, in contrast to previous studies of conventional, one-compartment, lactate-based PDFs.

Secondly, our study shows that biocompatibility is not just a function of a single characteristic, such as GDP content, and in large part due to other mechanisms. Over the years, the focus of research on biocompatibility has shifted, and most recently has centered on GDPs. As background, there are hundreds of reported GDPs from carbohydrates, and most of these have been known for more than 50 years. In recent discussions, the biocompatibility of PDFs has been largely evaluated according to levels of GDPs in isolation [44], without explicit consideration of other sources of toxicity. Our study highlights that both dextrose and GDPs are important for biocompatibility, but alone are not a complete surrogate or even complete explanation. The assessment of biocompatibility should consider all the candidates for toxicity in each PDF product under consideration. Given the residual variation of % ICG in our models after accounting for concentration of dextrose and GDPs, it is likely that a

large proportion of the basal cytotoxicity of PDFs is mediated by the relative presence of different, not-yet-defined or quantified compounds. For example, there are other glucose derivatives that may be detected in PDF (e.g., valeraldehyde [45]), and their impact on cytotoxicity and cell function remains to be elucidated.

Our study highlights that further work is still needed to improve the biocompatibility of PDFs. There are no long-term clinical studies that rank the relative importance of the various components of PDFs in relation to the risk of peritoneal membrane failure. The most helpful studies are those that report in vitro cytotoxicity, using models of basal or peritoneal mesothelial cell toxicity. Taken as a whole, the most cytotoxic components are accepted as being low pH, higher levels of cytotoxic GDPs, glucose, osmolality, and lactate [46]. Importantly, these effects are not singular, and there are interactions between them [34, 35]. In this study, the higher biocompatibility with Reguneal™ may be arising from the unique buffer formulation, although we did not specifically test this hypothesis. Notwithstanding, further research is needed into mechanisms for the cytotoxicity that are unassociated with either dextrose concentration or measured GDPs.

Our study used the L-929 continuous fibroblast cell line, which has been used extensively for screening toxicity of both chemical and biomaterials, including PDFs [19, 47–52]. In fact, cytotoxicity of GDPs in PDFs was first described using this assay [19], and later reproduced using viability, growth inhibition, cytokine release, wound healing and apoptosis on mesothelial cells [20, 53, 54]. We chose an exposure period of 72 h, to increase sensitivity of the system even though a normal PD dwell is 4–5 h. The L-929 assay itself is an artificial system which does not try to mimic what happens in the peritoneal cavity, i.e., according to exposure period or cell-type. Instead, the test measures basal cytotoxicity, defined as the effects on structures and functions that are common to all human cells, i.e., metabolism, transport processes and reproduction. The choice of cells for measuring basal cytotoxicity is less important, since basic cellular mechanisms are similar in both specialized and non-specialized cells. The correlation to in vivo toxicity is good, and the toxicity ranking of chemicals is almost the same regardless of cellular type [55]. For basal cytotoxicity, the choice of cells should be based on accuracy and reproducibility, and tests based upon established cell lines have the highest predictive value and are therefore the preferred procedure in all ISO standards for evaluating toxicity of medical devices intended for human use [Biological Evaluation of medical devices—Part 5: tests for in vitro cytotoxicity (ISO10993-5:2003)].

Biocompatibility of PDFs has also been tested using primary cultures of human peritoneal mesothelial cells (HPMC) instead of animal or transformed cell lines. Theoretically, this model has greater cell specificity, and better reflects the natural variability of cells in vivo. Experiments have been done to compare growth rates in the presence of GDP assessed using L-929-based models versus those using HPMC-based models. The response of L-929 fibroblasts is very similar to that of HPMC [47–49], although under certain experimental conditions, the exclusive use of L-929 cells may underestimate the full extent of GDP-associated toxicity. To our knowledge, however, there is no case where an impaired proliferation test with one method wrongly indicated bioincompatibility which later has turned out to be biocompatible with the other.

The in vitro approach in our study is not a replacement for in vivo clinical assessment, but is a call to action comparing Reguneal™ with other PDFs in Japan on peritoneal structure and function in the medium to long term. The prospective evaluation of different brands of PDF within dialysis registries has been rare, with the exception of the Hong Kong Renal Registry [56], which regularly benchmarks brands of PDF against each other. It is the sincere hope of the authors of this study that some kind of evaluation is undertaken within the governance of the Japanese Peritoneal Dialysis Outcomes and Practice Patterns Study or the Japanese Society of Dialysis Therapy Registry. Further studies involving clinical outcomes are sorely needed.

There are two important limitations of our study. The first is the small difference that we identified in cytotoxicity between Reguneal and other biocompatible fluids on the Japanese market. Most importantly, the size of this difference begs the question—does this small difference really matter for the peritoneal cavity of the patient? As alluded to above, further translational and clinical research is needed to test this question. The second important limitation is that the in vitro system we chose is not suitable for evaluating specific effects in the peritoneal cavity, such as cytokine release or other receptor-mediated effects, cell transition or organ-specific functions. The assay we have chosen measures effect on basal cellular mechanisms, and nothing else, and our conclusions are limited to only considerations around basal cytotoxicity.

Conclusions

In summary, Reguneal™ appears to be a biocompatible PDF in terms of basal cytotoxicity, although the results of our study should not be over-interpreted. The finding of inhibition of cell growth in our study is a prediction of in vivo toxicity, although this prediction would be more robust if it were reproducible in alternative in vitro systems. Studies involving HPMC continuous cell lines and primary cultures are being planned now. In addition, the findings of residual effects on biocompatibility that are independent of dextrose

and GDPs should lead to research, which is needed to identify all the characteristics of PDFs that influence biocompatibility, Finally, further translational and clinical research is necessary to determine the long-term effect of this PDF to the peritoneum in vivo.

Compliance with ethical standards

Conflict of interest All authors are employees of Baxter Healthcare.

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