Immunoprotection by polyethylene glycol in organ preservation solutions is not due to an immunomasking effect

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Abstract
Background: New organ preservation solutions that contain soluble polyethylene glycol (sPEG) molecules have been associated with reduction of acute rejection episodes.

Methods: In the present manuscript we tested in vitro whether sPEG molecules were able to mask donor alloantigens and reduce graft immunogenicity.

Results: Immunomasking effect was only evidenced when PEG molecules were covalently bound to donor cell surface.

Conclusion: We concluded that sPEG in preservation solution are unlikely to display ‘immunocamouflage’ property.

Keywords: immunocamouflage; ischemia/reperfusion; organ preservation solution; polyethylene glycols; transplantation

Introduction

The quality of organ preservation is becoming crucial for transplantation success in the context of the use of extended criteria donors [1–3]. New preservation solutions containing high molecular weight polyethylene glycol (PEG) molecules, such as IGL-1 (Institut Georges Lopez [4,5]), Cardiosol [6] and SCOT (solution for conservation of organs and tissues [7]) have been shown to efficiently protect organs from ischemia/reperfusion injury [5,8,9] while reducing the rate of acute rejection episode. Since PEG molecules are able to form colloidal structures creating a large excluding volume on the cell surface, it has been proposed that PEG could cover antigenic sites and attenuate grafts immunogenicity, a theory named ‘immunocamouflage’ [10]. Immunocamouflage has only been demonstrated so far for PEG molecules bound to the lymphocyte surface by a covalent link [11,12]. In this study, we tested in vitro the ability of soluble PEG (sPEG) contained in the organ conservation solutions to inhibit the immune responses.

Material and methods

Peripheral blood mononuclear cells (PBMCs) were prepared from healthy blood donors by centrifugation over a Ficoll gradient (Eurobio; France). All the monoclonal antibodies used were from Becton Dickinson Biosciences. The covalent binding of PEG molecules to the PBMC membrane was performed using cyanuric chloride-activated methoxy-polyethylene glycol (CmPEG) 5 kDa (Sigma chemical Co., France), or succinimidyl propionate-activated methoxy-polyethylene glycol (SPAmPEG) 5 kDa or 20 kDa (Nektar Therapeutics, USA.), in an alkaline phosphate buffer as described elsewhere [11].

Results

Effect of sPEG on cell surface antigen detection

We first analysed by flow cytometry the ability of soluble and bound PEGs to reduce the staining of PBMC surface antigens by monoclonal antibodies. Cell surface staining for CD3 and CD28 (Figure 1) but also CD1a, CD4, CD8, CD19, CD20, CD25, CD29, HLA I and HLA-DR (not shown) was not modified by the presence of increased concentrations of sPEGs (up to 24 mM, data not shown). In contrast, CmPEG 5 kDa (Figure 1) and SPAmPEG 5 and 20 kDa (not shown) lead to a dramatic decrease in cell surface fluorescence intensity for all tested antigens, in a dose-dependent manner.

Effect of sPEG on T cell proliferation

PBMCs were pre-incubated in medium containing sPEG (6, 20 or 35 kDa), CmPEG 5 kDa or SPAmPEG 5 and 20 kDa (not shown) lead to a dramatic decrease in cell surface fluorescence intensity for all tested antigens, in a dose-dependent manner.
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Fig. 1. PEGs are able to mask antigens on the PBMC membrane only when they covalently bind at the cell surface. Pegylated PBMCs, PBMCs treated with soluble PEG (Sigma) or untreated PBMCs were washed two times in PBS and incubated at $1.10^6$ cells/mL in staining medium, with or without soluble PEGs. Immunofluorescence intensity was analysed on a FACScan flow cytometer (BD Biosciences, France) with FlowJo software (Tree Star, Inc. USA). CD3 PE (A, B) and CD28 FITC (C) staining on PBMCs preincubated for 1 h in medium alone or soluble PEGs (2.4 mM PEG 6 kDa; 1.5 mM PEG 20 kDa; 0.3 mM PEG 35 kDa) or bound PEG (0.2 mM; 0.6 mM; 1.2 mM; 2.4 mM CmPEG 5 kDa). A: CD3 expression in dot blot representation. B, C: mean of fluorescent intensity (MFI) of CD3 PE (B) and CD28 FITC staining (C). Results are representative of three independent experiments.

Fig. 2. PBMC proliferation in response to SEB and PHA is not modified by soluble PEG molecules used in organs conservation but is dramatically decreased in a dose-dependent fashion by mPEGs. PBMCs ($2.5.10^5$/well) were stimulated with Staphylococcal Enterotoxin B (SEB, Sigma) (10 ng/mL) (A) or phytohemagglutinin (PHA, Sigma) (10 µg/mL) (B) or none for 72 h, after 1 h preincubation in medium alone or soluble PEGs (PEG 6 kDa 2.4 mM; PEG 20 kDa 1.5 mM; PEG 35 kDa 0.3 mM); bound PEGs: CmPEG5 kDa, SPAmPEG 5 kDa and SPAmPEG 20 kDa at 0.2 mM; 0.6 mM; 1.2 mM; 2.4 mM. After 3 days of incubation in triplicates in 96-wells plates, proliferation was measured by $^3$H-thymidine uptake and expressed as total cpm. Results are representative of 3 independent experiments.
and. Stimulator and responder cells, 1.10^5 cells/per well, were cocultured, in triplicate, in 96-well plates for 5 days, and proliferation was measured by [³H]-thymidine uptake (1 µCi/well; 12 h) and expressed as total c.p.m. Results are representative of three independent experiments.

phytohaemagglutinin. The sPEG had no effect on PBMC proliferation. In contrast, membrane-bound PEG readily inhibited PBMC proliferation in a dose-dependent manner (Figure 2).

The impact of PEG on the alloimmune response was then evaluated in a one-way MLR assay. Maintaining PBMCs in sPEG solutions at 37°C for several hours led to strong cell death. Therefore, the effect of a pre-incubation of the stimulator cells in sPEG solutions was compared to the effect of the covalent binding of PEG molecules to the surface of stimulator cells. Only membrane-bound PEG efficiently inhibited PBMC proliferation (Figure 3).

Discussion

Our results demonstrate that sPEG (including 1.5 mM PEG 20 kDa of the SCOT and 0.03 mM PEG 35 kDa of the IGL-1 solution) (i) did not reduce the accessibility of any of the cell surface antigens that we have tested, and (ii) did not modify lymphocyte response to a mitogen, a superantigen or alloantigens. In contrast, PEG molecules did display potent immunocamouflage properties when covalently bound to the cell membranes, as previously reported [11,12]. An obvious limitation of our study is the fact that these results were not obtained in an in vivo experimental model of organ transplantation. We believe that our simple in vitro model was appropriate to evaluate the immunocamouflage effect of sPEG of organ preservation solutions. Indeed, organs are kept in the PEG solution only during cold storage and washed before reperfusion, a situation closely mimicking our in vitro experimental setting, where cells were preincubated with PEG solutions before being used.

A parameter is, however, not adequately modelized in our study: the direct interactions of PEG with the proteins of the extracellular matrix [13]. This could explain the discrepancies between our results and the ones reported by Barrou et al. [7] that reported a potent immunocamouflage effect of the SCOT solution on pancreatic islets (known for their adhesion properties).

In conclusion, PEG molecules are able to preserve cells from immune recognition and activation only when they are covalently bound to the cell surface, a situation inappropriate for organ transplantation [14,15]. The benefits of using sPEG in organ preservation might be rather due to a better protection against ischaemic reperfusion injuries [4,16–18] leading to the reduction of the graft immunogenicity [3,4,19,20] than to a direct immunological effect linked to an ‘immunocamouflage’ phenomenon.

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Conflict of interest statement. None declared.

References

Preserved residual renal function is associated with lower oxidative stress in peritoneal dialysis patients

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Abstract

Background. Residual renal function (RRF) correlates with survival in peritoneal dialysis (PD). We investigated the association between oxidative stress and RRF in PD.

Methods. Adequacy of dialysis, total and free malondialdehydes (MDA), and lipid hydroperoxides (LHP) were obtained from 23 stable PD patients.

Results. Free MDA level decreased with total weekly Kt/V urea ($r = -0.51, P = 0.013$) and urinary Kt/V (KRU) ($r = -0.53, P = 0.009$), but not with peritoneal Kt/V. Similar results were found with LHP level. In multivariate analysis, total weekly Kt/V urea and KRU remained associated with free MDA and LHP, independently of gender, nutritional or inflammatory status, and peritoneal permeability.

Conclusion. A preserved RRF is associated with lower serum levels of lipid peroxidation products among PD patients.

Keywords: inflammation; lipid peroxidation; oxidative stress; peritoneal dialysis; residual renal function

Introduction

Residual renal function (RRF) correlates with survival in peritoneal dialysis (PD) patients [1]. In contrast, increasing peritoneal small-solute clearance has a neutral effect on outcome [2]. Thus, RRF seems to be physiologically more important than peritoneal clearance for patient outcome. The involved mechanisms are poorly understood. Considering the impact of oxidative stress (OS) on cardiovascular mortality in PD patients [3], we hypothesized that low RRF is associated with higher OS.

Methods

We enrolled 23 stable PD patients without any active infection. The patients’ characteristics are summarized in Table 1.

Peritoneal adequacy

The total weekly urea Kt/V and the normalized protein catabolic rate (nPCR) were assessed from the 24-h peritoneal effluent and urine collection (PD Adequest: Baxter Healthcare Corporation, McGaw Park, IL, USA). The contribution of total weekly urea Kt/V (total Kt/V) by PD (Kt/V PD) and RRF (KRU) were estimated separately. The peritoneal permeability was assessed by a peritoneal equilibration test.

Blood measurements

A blood sample was collected after an overnight fast for the analysis of serum concentration of urea, creatinine, albumin, pre-albumin and CRP by routine laboratory methods. Total and free malondialdehydes (MDA) were measured by

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