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The immunosuppressant drug cyclosporin A aggravates irradiation effects in endothelial cells

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Keywords

Cyclosporine A, endothelial cells, ICAM-1, apoptosis, VE-Cadherin, actin cytoskeleton.

Highlights

- CsA induces ICAM-1 overexpression through Toll-Like Receptor 4 (TLR4)-dependent NF- b activation
- CsA triggers apoptosis and rearrangement of the cytoskeleton and adherens junctions composed of VE-cadherin
- CsA aggravates irradiation-induced ICAM-1 overexpression, apoptosis, and rearrangement of the cytoskeleton and adherens junctions

Abstract

The immunosuppressant drug Cyclosporin A (CsA) has been widely used to prevent the development of Graft-versus-host-disease (GvHD) that can occur after transplantation, including allogeneic graft after accidental high-dose irradiation in humans. Here, we show that CsA alone stimulates ICAM-1 overexpression in human pulmonary microvascular endothelial cells (HPMECs) through Toll Like Receptor 4 (TLR4) and NF- κ B activation. In HPMECs, CsA treatment significantly worsened the overexpression of ICAM-1 induced by high-dose irradiation (15 Gy). This additive effect of CsA was also observed when ICAM-1 overexpression was induced by another pathway (Ca^{2+} entry) in macrovascular endothelial cells. In addition, CsA triggered apoptosis as well as rearrangement of the actin cytoskeleton and adherens junctions (VE-Cadherin) in microvascular endothelial monolayers. High dose irradiation triggered similar deleterious effects in endothelial monolayers and, again, CsA treatment strongly aggravated the effects of irradiation. Altogether, these results suggest that post-transplant CsA treatment may exacerbate the deleterious effects of irradiation on the endothelium.

Introduction

In the event of a nuclear accident, people may be exposed to high doses of irradiation, which can rapidly lead to a complete loss of white blood cells. Emergency treatments of this irradiation-induced haematopoietic syndrome include cytokines and growth factors, which may be efficient in the case of residual haematopoiesis, but bone marrow/haematopoietic stem cell transplantation may be necessary to allow recovery of haematopoiesis [7]. In case of allogeneic bone marrow/haematopoietic stem cell transplantation, immunosuppressive therapy is required to prevent graft-versus-host disease (GvHD)[5]. Among the available immunosuppressants, cyclosporin A (CsA, a calcineurin inhibitor) has been widely used in both experimental and clinical conditions to inhibit the release of pro-inflammatory cytokines, lymphocyte activation and proliferation, and development of GvHD [1;16]. In case of irradiation, CsA has also been used to prevent GvHD after haematopoietic stem cell transplantation [9;17].

However, in addition to its ability to prevent GvHD, CsA can induce endothelial dysfunction through multiple pathways including increased membrane expression of adhesion molecules and destabilisation of inter-endothelial junctions made of VE-Cadherin [4;13;14;21;22].

In addition to the haematopoietic syndrome, high dose irradiation is also known to induce endothelial dysfunction, including increased expression of adhesion molecules on the endothelial cell membrane (ICAM-1, V-CAM-1, P- and E-selectin), destabilisation of inter-endothelial junctions and endothelial cell apoptosis [11;15;19;20;25]. All of these effects of irradiation promote white blood cell migration into tissues, inflammation, development of oedema and can ultimately lead to hypoxia, alteration of tissues and organ failure [10;26].

As mentioned earlier, the deleterious vascular effects of CsA have been documented, but its effects on high-dose irradiated endothelial cells remain to be tested.

Here, we investigated the effect of CsA treatment on cultured human pulmonary microvascular endothelial cells (HPMECs) irradiated at high-dose. CsA alone triggered ICAM-1 overexpression through TLR4 and NF- κ B activation, apoptosis and alterations of the actin cytoskeleton and VE-Cadherin adherens junctions in endothelial cells. We then show that CsA significantly exacerbates all the deleterious effects of irradiation on endothelial cells, including overexpression of membrane ICAM-1, apoptosis and rearrangement of the actin cytoskeleton and adherens junctions. Therefore, our results suggest that the use of an alternative immunosuppressant may be preferable after irradiation at high dose.

Materials and methods

Material

The immunosuppressive drug Cyclosporin A (CsA), the Toll-Like Receptor 4 (TLR4) inhibitors (M62812 and Tak242) and the NF- κ B inhibitor BAY11-7085 were purchased from Tocris (Bristol, UK), while the TRPV4 agonist GSK1016790A was from Abcam (Cambridge, UK). The FITC-conjugated mouse anti-VE-Cadherin (CD144) antibody (clone 55-7H1, cat n° 580411) was from BD Pharmingen while Phalloidin 547 (FP-AZ0330) was from FluoProbes, Interchim, (Montluçon, France). DAPI Fluoromount-GTM mounting medium (cat n° 0100-20) was obtained from Southern biotech (Birmingham, AL 35209, USA). For flow cytometry analysis, all antibodies (BB515 mouse anti-human CD54 (ICAM-1, cat n° 564685), BUV737 mouse anti-human CD106 (VCAM-1, cat n° 565418), PE mouse anti-human CD62P (P-Selectin, cat n° 555524), APC mouse anti-human CD62E (E-Selectin, cat n° 551144)) and the FITC Annexin V/Propidium Iodide apoptosis detection kit were purchased from BD Biosciences.

Culture, irradiation and treatments of endothelial cells

Human pulmonary microvascular endothelial (HPMECs (Promocell, Switzerland)) were cultured in T25 flasks, 6-well plates (for cytometry measurements) or in 8-chamber Lab-Tek slides (for immunostainings) coated with attachment factor (Gibco). HPMECs were allowed to grow and reach confluence in endothelial cell growth medium MV2 (Promocell, Switzerland) containing endothelial cell growth medium supplement MV2 (Promocell, Switzerland) and Gentamicin/amphotericin (Gibco).

Human umbilical vein endothelial cells (HUVECs, Gibco) were also allowed to grow and reach confluence in T25 flasks or 6-well plates (for cytometry measurements) coated with

attachment factor (Gibco). Medium 200 containing low serum growth supplement and Gentamicin/amphotericin (Gibco) was used to cultivate HUVECs. For both HPMECs and HUVECs, cells from passage 3 to 6 were used in experiments.

Confluent HPMECs were irradiated at 15 Gy (dose-rate of 0,68 Gy/min) using an X-irradiator (SARRP (Xstrahl, UK)). High accuracy dosimetry was performed using small pieces of Gafchromic paper, as previously described [6].

In some experiments, HPMECs were treated with CsA 24 h after being irradiated, as indicated in the figures. In experiments from Fig. 1 and 2C, HPMECs were treated with CsA for 24 h before measurements, while TLR4 or NF- κ B inhibitors were added 10 min before treatment with CsA.

Immunostaining

HPMECs cultured in 8-chamber Lab-Tek slides were washed with DPBS, fixed with paraformaldehyde 2 % for 15 min and permeabilized with Triton X-100 (0.2 %) for 3 min at room temperature. Before being stained, HPMECs were incubated with 5 % bovine serum albumin in DPBS for 1 h at room temperature, in order to prevent non-specific labelling. HPMECs were then stained with FITC-conjugated mouse anti-CD144 (VE-Cadherin) antibody (1:5) and Phalloidin 547 (1:250) for 45 min at room temperature [3]. After extensive washing, coverslips were mounted on glass slides using DAPI Fluoromount-GTM. An OLYMPUS IX2-SLP microscope equipped with a Hamamatsu Orca-Flash4.0 V2 camera and NIS Elements Nikon software (version 4.51.00) was used to acquire images. All acquisition parameters were kept the same for all conditions in each experiment (laser power, exposition time).

Apoptosis assay

As described before, a FITC Annexin V/Propidium Iodide apoptosis detection kit (BD Biosciences) was used to measure apoptosis and necrosis in HPMECs [3]. Cells cultured in 6-well plates were detached and washed twice with cold PBS. HPMECs were then resuspended in binding buffer (100 μ l) and incubated with 5 μ l FITC Annexin V and 5 μ l Propidium Iodide (PI) for 15 min at 20 °C in the dark. After further addition of binding buffer, apoptosis of HPMECs was rapidly analysed by flow cytometry using a Becton Dickinson LSR II cytometer. FlowJo software was used to perform analysis, enabling to calculate the % of apoptotic cells (PI+, FITC Annexin V+).

Measurements of endothelial cell surface expression of adhesion molecules

Membrane expression of adhesion molecules (ICAM-1, VCAM-1, P-selectin and E-selectin) was measured by flow cytometry for HPMECs and HUVECs, as described previously [3]. Briefly, cells cultured in 6-well plates, were detached, washed and fixed with PBS containing 1 % paraformaldehyde. Cells were then washed and stained (30 min) with a four colours antibody panel composed of BB515 mouse anti-human CD54 (ICAM-1), BUV737 mouse anti-human CD106 (VCAM-1), PE mouse anti human CD62P (P-selectin) and APC mouse anti-human CD62E (E-selectin). After extensive washing with PBS, cell staining was analysed by flow cytometry using a Becton Dickinson LSR II cytometer. An anti-Mouse Ig, κ /Negative control compensation particles set (BD Biosciences) was used to set compensations in each experiment. FlowJo software was used to analyse all data, using Median Fluorescence Intensity (MFI) values to compare cell surface expression of ICAM-1, P-selectin, E-selectin and VCAM-1 in control and after cell irradiation/treatment.

Statistical analysis

Data are expressed as means \pm SEM. Statistical significance was evaluated with ANOVA tests, using GraphPad Prism software (San Diego, CA).

Results

Cyclosporin A increases the membrane expression level of ICAM-1 in human pulmonary microvascular endothelial cells.

Confluent HPMECs were incubated for 24 h with CsA (1, 10 and 30 μ M). After 24 h of incubation, cells were detached, fixed and membrane expression levels of adhesion molecules (ICAM-1, VCAM-1, E-selectin, P-selectin) were measured by flow cytometry. The membrane expression level of ICAM-1 was significantly increased after 24 h of CsA exposure at concentrations ≥ 10 μ M, as indicated by significant increases in ICAM-1 MFI (Fig. 1A). In contrast, the membrane expression levels of other adhesion molecules (VCAM-1, P-selectin and E-selectin) remained unchanged after 24 h of incubation with CsA, even at the highest concentration tested (Fig. 1A).

ICAM-1 gene expression is mainly controlled by the transcription factor NF- κ B [27]. To investigate whether the CsA-induced increase in ICAM-1 membrane expression is indeed related to NF- κ B activation, we tested the effect of BAY11-7085, a potent inhibitor of NF- κ B activity [12]. As shown in Fig. 1B, pre-incubation of HPMECs with BAY11-7085 (10 μ M) abolished the effect of CsA on ICAM-1 membrane expression, indicating that it relies on NF- κ B activation. Moreover, pre-treatment of HPMECs with the potent Toll-Like Receptor 4 (TLR4) inhibitors M62812 or Tak242 significantly reduced CsA-induced ICAM-1 overexpression, suggesting that the CsA-induced NF- κ B activation may be achieved through TLR4 activation by CsA (Fig. 1B) [18;23].

Cyclosporin A aggravates irradiation-induced overexpression of membrane ICAM-1 in pulmonary microvascular endothelial cells

HPMECs were irradiated at high dose (15 Gy, X-irradiation) and CsA was added to the cell culture medium 24 h after irradiation. Measurements were then performed 48 h after irradiation. The results in the Fig. 2A indicate that irradiation induced overexpression of ICAM-1, and also that the addition of CsA (at concentrations $\geq 10 \mu\text{M}$) to irradiated HPMECs triggered a significant increase in ICAM-1 expression compared to cells that had only been irradiated (Fig. 2A). In contrast, the plasma membrane expression of the other adhesion molecules investigated (E- and P-selectin, VCAM-1) was not significantly affected by irradiation or CsA (Fig. 2A). These experiments therefore suggest that the enhanced increase in ICAM-1 membrane expression observed in irradiated HPMECs exposed to CsA relies on the additive effects of CsA and irradiation.

Cyclosporin A increases the membrane ICAM-1 overexpression induced by cytosolic Ca^{2+} increases related to Ca^{2+} entry through TRP channels in macrovascular endothelial cells

As shown for HPMECs, only the membrane expression level of ICAM-1 was significantly increased in macrovascular endothelial cells (HUVECs) after 24 h exposure to CSA at concentrations $\geq 10 \mu\text{M}$ (Fig. 2B). We have recently shown that Ca^{2+} entry through Transient Receptor Vanilloid 4 channels (TRPV4) significantly increased the membrane expression level of ICAM-1 in macrovascular endothelial cells [3]. Here, preincubation of HUVECs with 30 nM GSK1016790A (a potent TRPV4 agonist [3]) induced an ICAM-1 overexpression that was significantly increased in the presence of CSA at concentrations $\geq 10 \mu\text{M}$ (Fig. 2B). These results indicate that an additive effect of CsA and the TRP channel agonist (GSK1016790A) is involved in ICAM-1 overexpression.

Cyclosporin A induces apoptosis in microvascular endothelial cells

HPMECs were incubated with CsA alone (1, 10 and 30 μ M) and apoptosis was evaluated 24 h after CsA addition, using a FITC Annexin V/Propidium Iodide apoptosis detection kit. Even at the lowest concentration tested (1 μ M), CsA triggered significant apoptosis in HPMEC monolayers (Fig. 3A and B). In contrast to what was observed for ICAM-1 overexpression, CsA-induced apoptosis was not inhibited by blocking TLR4 receptors with M62812 or Tak242 (Fig. 3C) [18;23]. Apoptosis was even significantly higher with M62812 treatment, suggesting that TLR4 blockade may promote apoptosis in HPMECs (Fig. 3C).

Cyclosporin A aggravates irradiation-induced apoptosis in microvascular endothelial cells

High-dose irradiation (15 Gy) triggered significant apoptosis of HPMECs when measured 72 h after irradiation, as indicated by the significant increases in the apoptotic cell fraction (Fig 3D). When a low CsA concentration (1 μ M) was added 24 h after irradiation, we observed a significant increase of the apoptotic fraction of HPMECs (Fig. 3D). Therefore, even low concentrations of CsA aggravate irradiation-induced apoptosis of HPMECs.

Effects of Cyclosporin A and irradiation on the cytoskeleton and adherens junctions in HPMECs

To investigate the effects of CsA and irradiation on the actin cytoskeleton and adherens junction in HPMECs, cells were stained with Phalloidin and anti-VE-Cadherin antibody. While actin appeared to be predominantly localised near the plasma membrane in control cells, high concentrations of CsA (10 and 30 μ M) induced an obvious rearrangement of the cytoskeleton, with a marked increase of peripheral intracellular F-Actin associated with the formation of Phalloidin-stained F-Actin stress fibres (Fig. 4). In addition, VE-Cadherin staining at plasma membrane appeared more winding after exposure of HPMECs to high

concentrations of CsA (30 μM), indicating a rearrangement of plasma membrane VE-Cadherin (Fig. 4).

High-dose irradiation (15 Gy) also triggered a rearrangement of the actin cytoskeleton with the formation of stress fibres, as high intensity F-Actin staining was more frequently observed near the plasma membrane and in the cell cytosol. However, in contrast to CsA treatment, no obvious alteration of VE-Cadherin staining was observed after irradiation with this protocol.

In HMPECs irradiated at 15 Gy and exposed to CsA, the formation of actin stress fibres and a VE-Cadherin rearrangement were observed, but these effects were enhanced when compared to cells exposed to CsA alone (Fig. 4). Indeed, even for the lowest CsA concentration tested (1 μM), the formation of actin stress fibres was increased in irradiated cells. Moreover, extensive intercellular gaps were highly visible in HMPECs irradiated at 15 Gy and exposed to CsA (10 and 30 μM), indicating that many cells in the endothelial monolayer were no longer joined (arrows in Fig. 4). Overall, these results indicate that the effects of CsA on the cytoskeleton and adherens junctions are additional to those of irradiation and also that CsA significantly aggravates the effect of irradiation on endothelial monolayers.

Discussion

Here, we investigated the effect of CsA, a widely used immunosuppressant, on human microvascular pulmonary endothelial cells exposed to high-dose X-ray.

We first show that exposure to CsA alone increased the membrane expression of ICAM-1 in both microvascular (HPMECs) and macrovascular (HUVECs) endothelial cells [2;4;21]. This effect of CsA was significantly reduced by potent inhibitors of both NF- κ B and TLR4 receptors, suggesting that the stimulation of ICAM-1 expression by CsA includes TLR4-dependent activation of NF- κ B in HPMECs, consistent with a recent study in murine endothelial cells [21].

High-dose irradiation of HPMECs (15 Gy) also triggered increased membrane expression of ICAM-1 and post-irradiation treatment of irradiated HPMECs with CsA significantly increased the stimulatory effect of high-dose X-irradiation on ICAM-1 expression. In fact, the effects of CsA and irradiation appeared to be additive with respect to ICAM-1 membrane expression. This additive effect of CsA was also observed when ICAM-1 expression was induced by a completely different mechanism (Ca^{2+} entry through TRPV4 channels) in another endothelial cell type (HUVECs), suggesting that it may occur regardless of the pathway triggering ICAM-1 overexpression [3].

Furthermore, both low concentrations of CsA and high-dose irradiation triggered significant apoptosis in HPMEC cultures and, as was the case for ICAM-1 expression, the pro-apoptotic

effect of CsA adds to that of irradiation regarding apoptosis of HPMECs. Finally, CsA significantly altered the actin cytoskeleton and adherens junctions, as evidenced by the formation of actin stress fibres and the rearrangement of VE-cadherin adherens junctions. Rearrangement of the actin cytoskeleton was also observed upon irradiation, with the formation of actin stress fibres. The effects of CsA on the cytoskeleton, adherens junctions and monolayer integrity were enhanced in irradiated HPMECs, indicating that CsA also significantly worsens the effect of irradiation on endothelial monolayers.

Overall, our results indicate that CsA alone induces ICAM-1 overexpression, apoptosis, actin stress fibre formation and adherens junction rearrangement, but more importantly that most of these deleterious effects are additional to those of irradiation and observed for CsA concentrations in the micromolar range, which corresponds to the CsA peak blood concentration in patients treated with this immunosuppressant [8].

Upon irradiation, leucocyte migration through the endothelium is facilitated by overexpression of ICAM-1, endothelial cell apoptosis and deregulation/rearrangement of the adherens junction, which ultimately promotes the development of excessive inflammation [10;11;15;19;20;25;26]. As CsA enhances all these effects, this suggests that post-irradiation treatment with CsA may significantly worsen the pro-inflammatory effects of irradiation, leading to increased tissue alteration and organ damages. In line with this hypothesis, it has recently been demonstrated in NOD scid gamma mice that CsA treatment combined with irradiation is hepatotoxic and even lethal [24]. Therefore, the additive deleterious effects of CsA on irradiated endothelial cells that we describe in the present work may partly explain the in vivo observations of these authors.

Conclusion

CsA has been widely used to prevent GvHD after transplantation, including bone marrow/haematopoietic stem cell transplantation required to restore haematopoiesis after to high-dose radiation exposure [1;9;16;17]. Therefore, the results presented here suggest that in case of bone marrow/haematopoietic stem cell graft/transplantation after accidental high-dose irradiation, the use of CsA as an immunosuppressant may aggravate the deleterious effect of irradiation on the endothelium, which could ultimately worsen inflammation, oedema and long-term tissue alterations caused by high-dose irradiation. Alternative immunosuppressant treatments may then be considered after bone marrow/haematopoietic stem cell graft/transplantation in case of irradiation.

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Disclosures

The authors declare no conflicts of interest.

Figure legends

Fig. 1. Effect of CsA on the surface expression of ICAM-1, VCAM-1, E-selectin and P-selectin in HPMECs. Membrane expression of adhesion molecules (ICAM-1, E-selectin, P-selectin and VCAM-1) was measured using flow cytometry 24 h after addition of CsA to the culture medium of HPMECs. A: Left: merged histograms of ICAM-1 staining for control HPMECs and cells treated with CsA at 1, 10 and 30 μ M for 24 h. Right: Averaged normalised

MFI values obtained for ICAM-1, E-selectin, P-selectin and VCAM-1 in CsA-treated HPMECs. Data on graphs represent compiled data from 9 experiments (* P <0.05, *** P <0.001). *B*: Left: merged histograms of ICAM-1 staining for control HPMECs and CsA-treated-cells (10 µM, 24 h) with or without BAY11-7085 (10 µM) or M62812 (30 µM). Right: Averaged normalised MFI for ICAM-1 in CsA-stimulated HPMECs (10 µM, 24h) treated or not with BAY11-7085 (10 µM), M62812 (30 µM) or Tak242 (1, 10 and 30 µM). Data on graphs represent compiled data from 8 experiments (** P <0.01, *** P <0.001).

Fig. 2. Effect of CsA treatment on irradiation- or Ca²⁺ entry-induced ICAM-1 membrane expression. Membrane expression of adhesion molecules (ICAM-1, E-selectin, P-selectin and VCAM-1) was determined by flow cytometry 48 h after 15 Gy-irradiation of HPMECs while CsA was added to the culture medium 24 h after cell irradiation. *A*: Top: Merged histograms of ICAM-1 staining in control and irradiated HPMECs (15 Gy) treated or not with CsA (1, 10 or 30 µM) 24 h after irradiation. Below: Averaged normalised MFI for ICAM-1, E-selectin, P-selectin and VCAM-1 stainings in irradiated HPMECs (15 Gy) treated or not with CsA (1, 10 and 30 µM) 24 h after irradiation. Effect of CsA alone (1, 10 and 30 µM) was also measured in these experiments. Data on graphs represent the average of 4 experiments (* P <0.05, ** P <0.01, *** P <0.001). *B*: Left: Effect of CsA on the surface expression of ICAM-1, E-selectin, P-selectin and VCAM-1 in HUVECs. Membrane expressions of ICAM-1, E-selectin, P-selectin and VCAM-1 were measured using flow cytometry 24 h after addition of CsA (1, 10 and 30 µM) to the culture medium of HUVECs. The graph represents averaged normalised MFI values obtained for ICAM-1, E-selectin, P-selectin and VCAM-1 in CsA-treated HUVECs. Data on graphs represent compiled data from 5 experiments (* P <0.05, *** P <0.001). Right: Averaged normalised MFI of ICAM-1 staining in GSK1016790A (GSK101, 30 nM)-stimulated HUVECs treated or not with CsA (1,

10 and 30 μM). Data on graphs represent compiled data from 4 experiments (** $P < 0.01$, *** $P < 0.001$).

Fig. 3. Effect of CsA and high-dose irradiation (15 Gy) on apoptosis of HPMECs. *A:* Flow cytometry analysis of HPMECs apoptosis in control and after incubation with CsA (1, 10 and 30 μM) for 24 h. Quadrants have been drawn on pictures in order to measure the % of apoptotic cells (PI+/Annexin V+) among HPMEC populations. *B:* Averaged proportion of apoptotic cells (PI+/Annexin V+) among HPMEC populations in control and after 24 h exposure to CsA (1, 10 and 30 μM). Data on graphs represent compiled data from 10 experiments (* $P < 0.05$, *** $P < 0.001$). *C:* Averaged proportion of apoptotic cells among HPMEC populations in control and after 24 h exposure to CsA (30 μM) with or without M62812 (30 μM) or Tak242 (30 μM) pre-treatment. Data on graphs represent the average of 4 experiments (** $P < 0.01$). *D:* Left: Flow cytometry analysis of HPMECs apoptosis in control and irradiated (15 Gy) cells treated or not with CsA (1 μM) 24 h after irradiation. Measurements were performed 72 h after irradiation. Quadrants have been drawn on pictures in order to measure the % of apoptotic cells (PI+/Annexin-5+) among HPMEC populations. Right: Mean averaged proportions of apoptotic cells among HPMEC populations in control and irradiated cells (15 Gy) treated or not with CsA (1 μM). Measurements were performed 72 h after irradiation, while CsA was added 24 h after irradiation. Data on graphs represent the average of 6 experiments (* $P < 0.05$, ** $P < 0.01$).

Fig. 4. Effect of CsA and high-dose irradiation (15 Gy) on the actin cytoskeleton and adherens junctions. Images represent stainings of F-Actin (red) and VE-Cadherin (green) in HPMEC monolayers in control and 24 h after irradiation (15 Gy) and/or CsA (1, 10 and 30 μM) treatment. Images presented are representative of 3 experiments. White arrows drawn on images indicate intercellular gaps. Scale bar: 10 μM .

References

Reference List

- [1] R. Arranz, E. Conde, F. Rodriguez-Salvanes, F.J. Pajuelo, R. Cabrera, M.A. Sanz, J. Petit, J. Bueno, J. Maldonado, J. Odriozola, J.G. Conde, S. Brunet, E. Carreras, A. Iriondo, J.M. Fernandez-Ranada, P. Marin, CsA-based post-graft immunosuppression: the main factor for improving outcome of allografted patients with acquired aplastic anemia. A retrospective survey by the Spanish Group of Hematopoietic Transplantation. *Bone Marrow Transplant.* 29 (2002) 205-211.
- [2] M.V. Badiwala, D. Guha, L. Tumiati, J. Joseph, A. Ghashghai, H.J. Ross, D.H. Delgado, V. Rao, Epidermal growth factor-like domain 7 is a novel inhibitor of neutrophil adhesion to coronary artery endothelial cells injured by calcineurin inhibition. *Circulation* 124 (2011) S197-S203.
- [3] K. Beddek, F. Raffin, D. Borgel, F. Saller, D. Riccobono, R. Bobe, F.X. Boittin, TRPV4 channel activation induces the transition of venous and arterial endothelial cells toward a pro-inflammatory phenotype. *Physiol Rep.* 9 (2021) e14613.
- [4] A. Carmona, M. Diaz-Ricart, M. Palomo, P. Molina, M. Pino, M. Rovira, G. Escolar, E. Carreras, Distinct deleterious effects of cyclosporine and tacrolimus and combined tacrolimus-sirolimus on endothelial cells: protective effect of defibrotide. *Biol.Blood Marrow Transplant.* 19 (2013) 1439-1445.
- [5] S.W. Choi, P. Reddy, Current and emerging strategies for the prevention of graft-versus-host disease. *Nat.Rev.Clin.Oncol.* 11 (2014) 536-547.
- [6] S.M. Dos, V. Paget, F. Trompier, G. Gruel, F. Milliat, Dosimetry for Cell Irradiation using Orthovoltage (40-300 kV) X-Ray Facilities. *J.Vis.Exp.*2021).
- [7] M. Drouet, F. Herodin, Radiation victim management and the haematologist in the future: time to revisit therapeutic guidelines? *Int.J.Radiat.Biol.* 86 (2010) 636-648.
- [8] S. Gerull, C. Arber, C. Bucher, A. Buser, A. Gratwohl, J. Halter, D. Heim, A. Tichelli, M. Stern, Cyclosporine levels and rate of graft rejection following non-myeloablative conditioning for allogeneic hematopoietic SCT. *Bone Marrow Transplant.* 46 (2011) 740-746.
- [9] M. Guo, Z. Dong, J. Qiao, C. Yu, Q. Sun, K. Hu, G. Liu, L. Wei, B. Yao, Q. Man, X. Sun, Z. Liu, Z. Song, C. Yu, Y. Chen, Q. Luo, S. Liu, H.S. Ai, Severe acute radiation syndrome: treatment of a lethally ⁶⁰Co-source irradiated accident victim in China with HLA-mismatched peripheral blood stem cell transplantation and mesenchymal stem cells. *J.Radiat.Res.* 55 (2014) 205-209.
- [10] D.E. Hallahan, S. Virudachalam, Intercellular adhesion molecule 1 knockout abrogates radiation induced pulmonary inflammation. *Proc.Natl.Acad.Sci.U.S.A* 94 (1997) 6432-6437.

- [11] M. Heckmann, K. Douwes, R. Peter, K. Degitz, Vascular activation of adhesion molecule mRNA and cell surface expression by ionizing radiation. *Exp.Cell Res.* 238 (1998) 148-154.
- [12] X. Hu, W.E. Janssen, L.C. Moscinski, M. Bryington, A. Dangsupa, N. Rezai-Zadeh, B.A. Babbitt, K.S. Zuckerman, An IkappaBalpha inhibitor causes leukemia cell death through a p38 MAP kinase-dependent, NF-kappaB-independent mechanism. *Cancer Res.* 61 (2001) 6290-6296.
- [13] T. Ikezoe, J. Yang, C. Nishioka, G. Honda, M. Furihata, A. Yokoyama, Thrombomodulin protects endothelial cells from a calcineurin inhibitor-induced cytotoxicity by upregulation of extracellular signal-regulated kinase/myeloid leukemia cell-1 signaling. *Arterioscler.Thromb.Vasc.Biol.* 32 (2012) 2259-2270.
- [14] T. Ikezoe, J. Yang, C. Nishioka, K. Umezawa, A. Yokoyama, Thrombomodulin blocks calcineurin inhibitor-induced vascular permeability via inhibition of Src/VE-cadherin axis. *Bone Marrow Transplant.* 52 (2017) 245-251.
- [15] P.N. Kouam, G.A. Reznicek, I.A. Adamietz, H. Buhler, Ionizing radiation increases the endothelial permeability and the transendothelial migration of tumor cells through ADAM10-activation and subsequent degradation of VE-cadherin. *BMC.Cancer* 19 (2019) 958.
- [16] Z.Y. Li, Q. Wu, Z. Yan, D. Li, X. Pan, T. Qiu, K. Xu, Prevention of acute GVHD in mice by treatment with *Tripterygium hypoglaucum* Hutch combined with cyclosporin A. *Hematology.* 18 (2013) 352-359.
- [17] H. Nagayama, K. Misawa, H. Tanaka, J. Ooi, T. Iseki, A. Tojo, K. Tani, Y. Yamada, H. Kodo, T.A. Takahashi, N. Yamashita, S. Shimazaki, S. Asano, Transient hematopoietic stem cell rescue using umbilical cord blood for a lethally irradiated nuclear accident victim. *Bone Marrow Transplant.* 29 (2002) 197-204.
- [18] M. Nakamura, Y. Shimizu, Y. Sato, Y. Miyazaki, T. Satoh, M. Mizuno, Y. Kato, Y. Hosaka, S. Furusako, Toll-like receptor 4 signal transduction inhibitor, M62812, suppresses endothelial cell and leukocyte activation and prevents lethal septic shock in mice. *Eur.J.Pharmacol.* 569 (2007) 237-243.
- [19] F. Paris, Z. Fuks, A. Kang, P. Capodiceci, G. Juan, D. Ehleiter, A. Haimovitz-Friedman, C. Cordon-Cardo, R. Kolesnick, Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. *Science* 293 (2001) 293-297.
- [20] S. Quarmby, P. Kumar, S. Kumar, Radiation-induced normal tissue injury: role of adhesion molecules in leukocyte-endothelial cell interactions. *Int.J.Cancer* 82 (1999) 385-395.
- [21] R. Rodrigues-Diez, C. Gonzalez-Guerrero, C. Ocana-Salceda, R.R. Rodrigues-Diez, J. Egido, A. Ortiz, M. Ruiz-Ortega, A.M. Ramos, Calcineurin inhibitors cyclosporine A and tacrolimus induce vascular inflammation and endothelial activation through TLR4 signaling. *Sci.Rep.* 6 (2016) 27915.
- [22] C.L. Schlichting, W.D. Schareck, M. Weis, Dendritic cell adhesion is enhanced on endothelial cells preexposed to calcineurin inhibitors. *J.Cardiovasc.Pharmacol.* 46 (2005) 250-254.
- [23] K. Takashima, N. Matsunaga, M. Yoshimatsu, K. Hazeki, T. Kaisho, M. Uekata, O. Hazeki, S. Akira, Y. Iizawa, M. Ii, Analysis of binding site for the novel small-molecule TLR4 signal

transduction inhibitor TAK-242 and its therapeutic effect on mouse sepsis model. *Br.J.Pharmacol.* 157 (2009) 1250-1262.

- [24] L. Walcher, C. Muller, N. Hilger, A. Kretschmer, L. Stahl, S. Wigge, J. Rengelshausen, A.M. Muller, S. Fricke, Effect of combined sublethal X-ray irradiation and cyclosporine A treatment in NOD scid gamma (NSG) mice. *Exp.Anim* 68 (2019) 1-11.
- [25] H. Wang, R.C. Segaran, L.Y. Chan, A.A.M. Aladresi, A. Chinnathambi, S.A. Alharbi, G. Sethi, F.R. Tang, Gamma Radiation-Induced Disruption of Cellular Junctions in HUVECs Is Mediated through Affecting MAPK/NF-kappaB Inflammatory Pathways. *Oxid.Med.Cell Longev.* 2019 (2019) 1486232.
- [26] J.P. Williams, W.H. McBride, After the bomb drops: a new look at radiation-induced multiple organ dysfunction syndrome (MODS). *Int.J.Radiat.Biol.* 87 (2011) 851-868.
- [27] J. Xue, P.B. Thippegowda, G. Hu, K. Bachmaier, J.W. Christman, A.B. Malik, C. Tiruppathi, NF-kappaB regulates thrombin-induced ICAM-1 gene expression in cooperation with NFAT by binding to the intronic NF-kappaB site in the ICAM-1 gene. *Physiol Genomics* 38 (2009) 42-53.