15-deoxy-Δ^{12,14}-PGJ2 induces cell cycle arrest and apoptosis of haematopoietic progenitors

Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-activated transcriptional factor that belongs to the nuclear receptor superfamily. Agonists that bind to PPARγ and stimulate its transcriptional activity include synthetic compounds (thiazolidinediones that are used for treating type 2 diabetes) and endogenous lipids, such as prostaglandin 15-deoxy-Δ^{12,14}-prostaglandin J2 (15d-PGJ2) (Takano & Komuro, 2009). Unlike other prostaglandins, there is no specific prostaglandin synthase leading to 15d-PGJ2 production and no specific 15d-PGJ2 receptor has been identified to date. Instead, 15d-PGJ2 has been shown to act via prostaglandin D2 receptors (DP1 and DP2) and through interaction with intracellular targets. Several studies showed that 15d-PGJ2 possesses not only antiinflammatory but also antineoplastic activity in human cancer of various origins (Grommes et al., 2004) and therefore is considered a potential useful therapeutic agent for the treatment of cancer. Although PPARγ has been detected in a variety of different haematopoietic cell types, including CD34+ cells, monocytes/macrophages, lymphocytes and megakaryocytes (Greene et al., 2000; Akbiyik et al., 2004), its role in normal haematopoiesis is still not fully elucidated. While 15d-PGJ2 delays primary erythroid progenitor maturation and proliferation (Nagasawa et al., 2005) it enhances megakaryocyte maturation and platelet generation (O’Brien et al., 2008). To further understand the role of 15d-PGJ2 in the process of haematopoiesis, this study analysed the effect of this prostaglandin on haematopoietic progenitor’s growth.

To evaluate the growth of granulocyte-macrophage colony-forming units (CFU-GM), granulocytic, erythroid,
macrophage, megakaryocyte CFU (CFU-GEMM), erythroid burst-forming units (BFU-E) and megakaryocyte CFU (CFU-MK). CD34⁺ cells were purified from human umbilical cord blood by magnetic immunoselection (Miltenyi, Bergisch Gladbach, Germany) (Negrotto et al., 2006) and seeded in either methylcellulose (MethoCult-H4434; StemCell Technologies, Vancouver, Canada) or collagen-based medium (MegaCult-C-4950; StemCell Technologies) in the presence or absence of 15d-PGJ₂ (Biomol, Plymouth Meeting, PA, USA). CFUs were identified by morphology or by anti-CD41 antibody and alkaline phosphatase detection system respectively at 10–12 d post plating.

The treatment of CD34⁺ cells with 15d-PGJ₂ significantly decreased BFU-E, CFU-GM, CFU-GEMM and CFU-MK formation (Fig 1A). In agreement with these results, CD34⁺ cell expansion stimulated with stem cell factor (SCF), interleukin (IL)-3, erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) or thrombopoietin (TPO) (all from Sigma, St Louis, MO, USA) was also impaired by 15d-PGJ₂ (Fig 1B). Altogether, these data demonstrated that 15d-PGJ₂ exerts a nonspecific inhibitory activity on haematopoietic progenitor growth.

Kinetics studies showed that reduction in cell proliferation and viability assessed by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Sigma) was already significant 3 d after 15d-PGJ₂ treatment (Fig 1C).

To investigate the mechanisms underlying the anti-proliferative effect of 15d-PGJ₂, we next analysed cell cycle distribution and apoptosis. Exposure of CD34⁺ cells to 15d-PGJ₂ resulted in a significant decrease in the proportion of cells in both G2 and S phases (propidium iodide (PI) staining; Fig 1D). Furthermore, treatment of cells with 15d-PGJ₂ increased the percentage of apoptotic cells in a concentration-dependent manner (Fig 2A) The induction of apoptosis in CD34⁺ cells cultured with 15d-PGJ₂ was confirmed by the increased Annexin V-FITC binding (Sigma) (7 ± 2%, 15 ± 1%* and 55 ± 4%* for 0, 10 and 20 µmol/l of 15d-PGJ₂ respectively, n = 4, *P < 0.05 versus vehicle) (Fig 2B). Thus, our results showed that the inhibitory activity of 15d-PGJ₂ on cell growth was associated with induction not only of cell cycle arrest, but also programmed cellular death.

To gain deeper insight into the molecular mechanism associated with the apoptotic effect of 15d-PGJ₂, we examined the involvement of the mitochondrial pathway. Fluorescence-activated cell sorting (FACS) studies showed that induction of apoptosis was associated to a down-regulation of the anti-apoptotic protein Bcl-xL (Southern Biotech, Birmingham, AL, USA), (Control = 94 ± 1% and 15d-PGJ₂ = 61 ± 2% of positive cells, n = 3, P < 0.05, Fig 2C). The decrease in Bcl-xL mediated by 15d-PGJ₂ resulted in the loss of mitochondrial membrane integrity. Exposure of CD34⁺ cells to 15d-PGJ₂ showed a sharp decrease in ΔΨₘ, reflected by a lower red/green fluorescence ratio of JC-1 staining (BD Pharmingen, San

Fig 2. 15d-PGJ₂ induces apoptosis of CD34⁺ cells by a PPARγ-independent mechanism. CD34⁺ cells were treated with 15d-PGJ₂ or vehicle for 48 h and apoptosis induction was evaluated by (A) Acridine orange and ethidium bromide staining and fluorescence microscopy (*P < 0.05 versus vehicle, n = 5) and (B) Annexin-V binding, PI staining and flow cytometry. Apoptotic pathway was evaluated by flow cytometry analysing: (C) Bcl-xl expression and (D) caspase-3 activation. Histograms show one representative experiment (n = 3 in duplicate). (E) CD34⁺ cells were preincubated for 60 min in the absence or presence of GW9662 (5 µmol/l), NAC (1 mmol/l) or BAY 11-7082 (5 µmol/l) before addition of 15d-PGJ₂ (20 µmol/l). Apoptosis was evaluated after acridine orange and ethidium bromide staining (n = 5 in duplicate). *P < 0.05 versus control, #P < 0.05 versus BAY 11-7082 or GW9662 and & P < 0.05 versus 15d-PGJ₂ alone.

Delineate the possible mechanism by which 15d-PGJ_2 induced degenerative diseases. Treatment of cancer and various inflammatory and neurogenerative diseases account, considering that this drug is emerging as potential inhibitors of PPAR\(_g\) activation. In fact, inhibition of nuclear factor (NF)-\(\kappa\)B activation, as well as the capacity of 15d-PGJ_2 to generate reactive oxygen species (ROS), have been involved as the death pathways triggered by this molecule (Ray et al, 2006). To delineate the possible mechanism by which 15d-PGJ_2 induced cell death in haematopoietic progenitors, GW9662 (Calbiochem, San Diego, CA, USA), BAY 11-7082 (Biomol) and \(N\)-Acetyl-L-cysteine (NAC) (Sigma) were used as specific inhibitors of PPAR\(_g\), NF-\(\kappa\)B and ROS generation respectively. Figure 2E shows that while GW9662 had no effect, preincubation with BAY 11-7082 increased and NAC completely prevented the cytotoxic effect of 15d-PGJ_2. These data indicated that 15d-PGJ_2 induced apoptosis of CD34\(^+\) cells is independent of PPAR\(_g\) and that generation of ROS appears to be a key mediator of this prostaglandin cytotoxic effect. Interestingly, abnormal accumulation of ROS has been implicated in the pathogenesis of haematological diseases, such as ataxia telangiectasia and Fanconi anaemia (Cumming et al, 2001; Ito et al, 2004). Haematopoiesis is a complex process in which haematopoietic stem cells proliferate, differentiate and generate a large number of lineage-committed blood cells. An adjusted balance between self-renewal and differentiation is necessary to maintain an adequate number of haematopoietic progenitors and mature blood cells. Our results, showing for the first time a negative regulatory effect of 15d-PGJ_2 on haematopoietic progenitor survival, should be taken into account, considering that this drug is emerging as potential treatment of cancer and various inflammatory and neurodegenerative diseases.

**Acknowledgements**

This work was supported by grants from ANPCYT (PICTs 1990, 25754) and Fiorini Foundation.

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**References**


**Keywords:** CD34\(^+\) cells, 15-deoxy-Delta12,14-prostaglandin J2, Peroxisome proliferator–activated receptor \(\gamma\), apoptosis.

First published online 18 September 2009
doi:10.1111/j.1365-2141.2009.07910.x

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