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Development of B Cells and Erythrocytes Is Specifically Impaired by the Drug Celastrol in Mice

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Abstract

Background: Celastrol, an active compound extracted from the root of the Chinese medicine “Thunder of God Vine” (Tripterygium wilfordii), exhibits anticancer, antioxidant and anti-inflammatory activities, and interest in the therapeutic potential of celastrol is increasing. However, described side effects following treatment are significant and require investigation prior to initiating clinical trials. Here, we investigated the effects of celastrol on the adult murine hematopoietic system.

Methodology/Principal Findings: Animals were treated daily with celastrol over a four-day period and peripheral blood, bone marrow, spleen, and peritoneal cavity were harvested for cell phenotyping. Treated mice showed specific impairment of the development of B cells and erythrocytes in all tested organs. In bone marrow, these alterations were accompanied by decreases in populations of common lymphoid progenitors (CLP), common myeloid progenitors (CMP) and megakaryocyte-erythrocyte progenitors (MEP).

Conclusions/Significance: These results indicate that celastrol acts through regulators of adult hematopoiesis and could be used as a modulator of the hematopoietic system. These observations provide valuable information for further assessment prior to clinical trials.

Introduction

Tripterygium wilfordii, an ivy-like vine also known as the “Thunder God Vine”, has been used as natural medicine in China for hundreds of years [1]. Celastrol, a quinone methide triterpenoid, was identified to be one of its active components. As root extract or purified compound, its remarkable anti-inflammatory ability has been demonstrated in animal models of different inflammatory diseases including asthma [2], Crohn’s disease [3], and neurodegenerative disorders [4,5]. Purified celastrol showed anticancer activity, in vivo in various tumor models of melanoma [6], prostate [7] and breast [8] cancer, as well as in vitro on leukemia cell lines [9,10,11], suggesting its use as a cancer therapeutic.

However, multiple side effects have been reported, including leukopenia, thrombocytopenia and anemia [12]. These adverse reactions are transient and recovery is usually complete upon removal of the drug. The molecular bases of the therapeutic and side effects are not well understood. Therefore, to advance celastrol as a therapeutic and prevent side effects, its toxicity and mechanism of action need to be revealed.

In the present study, we investigated the effects of celastrol on the hematopoietic system of adult mice with the aim of describing the immediate effects of celastrol on different mature and progenitor hematopoietic cell populations. We observed significant alterations of stem cells, progenitors and fully differentiated cell populations in peripheral blood (PB), bone marrow (BM), spleen and peritoneal cavity (PerC). These data indicate significant hematotoxicity, and suggest differential effects of celastrol on specific hematopoietic subsets. Understanding these effects will better enable the use of this potential therapeutic agent and will identify new clinical applications.

Materials and Methods

1- Mice and chemical treatment

BALB/c mice, 8- to 10-weeks-old, were purchased from Jackson Labs. All mice were maintained in the Animal Facility at Stanford University School of Medicine. All experiments were conducted under strict adherence to institutional guidelines, as approved by the Animal Care and Use Committee at Stanford University (aplac #12325). Mice received daily intraperitoneal (IP) injections (200 μL) of either celastrol (Cayman Chemical, purity ≥ 98%) diluted solutions (0.01, 0.1, 1 or 5 mg/kg/day), or...
carrier only (PBS with 5% DMSO) as a control, over the course of four days.

2- Tissue preparation and flow cytometry

Tissues were harvested the day following the last injection as previously described [13]. Cells were counted and stained with antibodies for phenotyping. For mature population analyses, cell suspensions were preincubated with anti–CD16/CD32 mAb to block FcγRII/III receptors and stained with the following fluorochrome-conjugated mAb: FITC-labeled anti-CD71; PE-labeled anti–Ter119, PECy5.5-labeled anti-CD5; PECy5.5-labeled anti-CD19; PECy7-labeled anti–IgM; APC-Cy5.5-labeled anti-IgD; APC-Cy7-labeled anti-CD11b and Pacific Blue–labeled anti–Gr-1, and Violet Green-labeled LIVE/DEAD®. For progenitors analyses, cells were stained with FITC-labeled anti-CD34, PE-labeled anti-Scal, APC-labeled anti-c-Kit, PECy7-labeled anti–II7Ra, APC-Cy7-labeled anti-CD16/32 and PerCP-Cy5.5-labeled lineage antibody cocktail. Antibodies were either purchased (Invitrogen and BD) or conjugated in the Herzenberg laboratory. Cells were analyzed on LSRII (BD). Data were analyzed with FlowJo software (TreeStar). In the carrier only treated-animals (PBS with 5% DMSO) there were no detectable changes in the levels of CD expression.

3- Statistical analyses

Quantitative data are expressed as the mean ± SEM. Statistical significance was assayed using a non-parametric Mann Whitney test (n = 10 mice. *p<0.05; **p<0.01; ***p<0.001).

Results and Discussion

1- Changes in peripheral blood parameters in celastrol treated-mice

To investigate the effects of celastrol on the hematopoietic system, mice received daily IP injections (0.01, 0.1, 1 or 5 mg/kg/day) over the course of 4 days, and tissues were harvested the day following the last injection. Values are percent of control. RBC: red blood cells; HGB: Hemoglobin; HCT: hematocrit; WBC: white blood cells. Mean ± SEM, n = 10.
Analysis of the different sub-populations of red cell progenitors was performed as described [21,22]. For CD71 and Ter119 expressions) from bone marrow, spleen and peritoneal cavity of control (left) and celastrol treated-mice (right) (n = 10). FACS-analysis of the different sub-populations of red cell progenitors was performed as described [21,22].

The alterations affecting the development of B cells and erythrocytes were also found in BM as well as in spleen and PerC (Figure 2B and 2C). In the PerC, the B-1 population [13] was highly affected (13.5-fold decrease; ***p<0.001) (Figure 2B). Celastrol did not appear to be directly cytotoxic in our study, as we did not observe decreases in the cellularity in any of the tested organs (Figure 1B). This is in agreement with a study describing no decrease in cell viability and no evidence of increased apoptosis of CD34+ human BM cells treated with extract of T. wilfordii [16]. Moreover, using colony-forming cell assays, it was demonstrated that the extracted compound directly blocks the ability of very early human hematopoietic multi-lineage, as well as lineage-specific committed, human hematopoietic progenitor cell to respond to growth factors and form colonies [16]. Thus we anticipate that the erythrocytic and B-lymphoblastic suppression described in our present study may be

Figure 2. Celastrol treatment results in multiple defects in mature lineages. (A) Representative FACS analysis of mature lineages from peripheral blood of control (left) and celastrol treated-mice (right). Mice received four consecutive daily intraperitoneal injections and peripheral blood cells were harvested the day following the last injection, processed and analyzed as described in Materials and Methods. The percentages shown correspond to raw data numbers. Data shown are representative of 10 mice. (B) Representative FACS analysis of total B cells (gated as LIVE/DEAD+, CD5+, CD19+, and analyzed for IgD and IgM expression) from bone marrow, spleen and peritoneal cavity of control (left) and celastrol treated-mice (right) (n = 10). FACS-analysis of the different sub-populations of B lymphoid progenitors was performed as described [13,20]. MZ B cells: Marginal Zone B cells. (C) Representative FACS analysis of total red cells (gated as LIVE/DEAD+, CD5+, CD19+, CD11b, Gr-1+, Sca-1+, and analyzed for CD71 and Ter119 expressions) from bone marrow, spleen and peritoneal cavity of control (left) and celastrol treated-mice (right) (n = 10). FACS-analysis of the different sub-populations of red cell progenitors was performed as described [21,22].

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Figure 3. Celastrol treatment results in multiple defects in BM progenitors. Representative FACS analysis of Megakaryocyte-Erythrocyte Progenitors (MEP), Common Myeloid Progenitors (CMP), Granulocyte-Monocyte Progenitors (GMP), LSK CD34+ (Lin− Sca-1+ c-Kit+ CD34+) cells and Common Lymphoid Progenitors (CLP) from bone marrow of control (left) and celastrol treated-mice (right) (n = 10). Cells were harvested from animals treated as described in Figure 2 and percentages shown correspond to raw data numbers. FACS-analysis of the different sub-populations of multipotent progenitors was performed as described [23].

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due to a loss of B and red cell regenerative potential from pluripotent cells exposed to celastrol.

3- Celastrol treatment results in multiple defects in bone marrow progenitors

Therefore, we examined the distribution of the different progenitors in the BM from celastrol-treated mice (Figure 3). We observed a 1.3-fold increase in the number of LSK CD34+ cells (Lin− Sca1− c-Kit+ CD34−), when compared to control mice (**p<0.01). We also showed decreases in the number of Common Myeloid Progenitors (GMPs) (2-fold; ***p<0.01) and Megakaryocyte-Erythrocyte Progenitors (MEPs) (1.7-fold; **p<0.01), whereas the number of Granulocyte-Monocyte Progenitors (GMPs) increased (1.9-fold; *p<0.01). This suggests a potential priming of celastrol-treated CMPs towards GMPs. Finally, the number of Common Lympoid Progenitors (CLPs) was significantly decreased (17-fold; **p<0.001) after celastrol treatment.

These results show that celastrol specifically impairs the development of B cells and erythrocytes in PB, BM, spleen and PerC. Thus, a potential use of the drug could be to modulate the hematopoietic system suggest that there may be several molecular targets, and this will need to be resolved for a more complete understanding of both the desired, and the adverse, effects of celastrol as a potential therapeutic agent. It is now apparent that the adverse effects of celastrol on the hematopoietic system need to be thoroughly evaluated prior to the initiation of further clinical studies.

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Author Contributions
Conceived and designed the experiments: SK EG. Performed the experiments: SK EG. Analyzed the data: SK EG. Contributed reagents/materials/analysis tools: SK EG LH CC. Wrote the paper: SK. Revised the manuscript: EG LH CC.

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