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Stem cells in myelotoxicity

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ABSTRACT

Myelotoxicity describes bone marrow failure due to adverse effect of xenobiotic on hematopoiesis. Hematopoiesis is a complex system in which pluripotent hematopoietic stem cells (PHSCs) differentiate into many highly specialized circulating blood cells involving the interaction of many cell types as well as the interaction of local and systemic growth factors. With respect to blood cell formation, two functional systems must be considered: the hematopoietic stem cells (PHSCs) and the progenitor cells, on one hand, and the stromal cells, which constitute the hematopoietic environment niche, on the other hand. There are three types of assays for hematopoietic progenitor clonogenic assays useable in myelotoxicology: CFU-GM assay for Colony Forming Unit Granulocyte and Macrophage, BFU-E assay for Burst Forming Unit Erythroid, and CFU-MK assay for Colony Forming Unit Megakaryocyte from several species as well as from murine as from mammalian and human.

Clonogenic assays have been used to detect myelotoxicity induced by chemicals, drug, food and environmental contaminants. Designs and applications are described in this review.

1. Hematopoietic stem cell and myelotoxicity

Hematopoiesis is a complex system in which pluripotent hematopoietic stem cells (PHSCs) differentiate into many highly specialized circulating blood cells involving the interaction of many cell types as well as the interaction of local and systemic growth factors. With respect to blood cell formation, two functional systems must be considered: the hematopoietic stem cells (PHSCs) and the progenitor cells, on one hand, and the stromal cells, which constitute the hematopoietic environment niche, on the other hand.

Adult stem cells are primitive cells that undergo asymmetric division, thereby giving rise to one clonogenic, self-renewing cell and one cell able to undergo multipotent differentiation. Hematopoietic stem cell is a multipotent stem cell; it can form multiple lineages that constitute an entire tissue. Progenitor cell is a generic term for any dividing cell with the capacity to differentiate and includes putative stem cells in which self-renewal has not yet been demonstrated. Niche cellular microenvironment provides support and stimuli necessary to sustain self-renewal (Smith, 2006).

Stem cells are characterized by their ability to self-renew and produce numerous types of differentiated daughter cells; two properties that enable them to maintain tissues. Self-renewal activity and multi-lineage differentiation potential of tissue stem cells are required to supply functionally differentiated progenies as normal tissue turn over.

Adult stem cells have been isolated from adult tissue, umbilical cord blood and other non-embryonic sources, and can transform into many tissues and cell types in response to pathophysiological stimuli. Clinical applications of adult stem cells and progenitor cells have potential in the regeneration of blood cells, skin, bone, cartilage and heart muscle, and may have potential in degenerative diseases. Multi-pluripotent adult stem cells can change their phenotype in response to trans-differentiation or fusion and their therapeutic potential could include therapies regulated by pharmacological modulation, for example mobilising endogenous stem cells and directing them within a tissue to stimulate regeneration.

The integrity of these two systems and their close cellular association are factors essential for maintaining normal hematopoiesis. A limited number of hematopoietic stem cells (PHSCs) are characterized by multipotential and self-renewing capacities, giving rise to progenitors of different lineages. These committed progenitors proliferate rapidly to increase the tissue mass. They differentiate, in their particular microenvironment, into mature blood cells in response to humoral growth factors and local cytokines. *In vitro*, the progenitors give rise to phenotypically distinct colonies of differentiated cells in the presence of tissue culture media containing specified mixtures of cytokines for each particular lineage.

Myelotoxicity describes bone marrow failure due to adverse effect of xenobiotic on hematopoiesis.

Its rapid rate of cell renewal and differentiation makes the hematopoietic system a susceptible target for xenobiotic toxicity:

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xenobiotics may interfere with cell proliferation and differentiation. The term "bone marrow failure" refers to a reduction in circulating blood cells resulting primarily from the failure of bone marrow hematopoietic stem cells to produce mature circulating blood cells. There are two major groups of bone marrow failure, aplastic anemia, where the failure lies in the pluripotent stem cell, and single cytopenia, where the failure possibly lies in one or the other of the committed cell lines. Most of the bone marrow failures are characterized by inadequate production of blood cells.

Stem cells are an important new tool for developing unique, *in vitro* model systems to test drugs and chemicals and a potential to predict or anticipate toxicity in humans as in cardiotoxicity, hepatotoxicity, embryotoxicity (Davila et al., 2004). Use of hematopoietic stem cells in *in vitro* myelotoxicity has a long history.

2. Myelotoxicity hazard identification and characterization

2.1. In vivo

Standard animal toxicology uses inhalation or ingestion of dose-feed preparations to achieve exposure. In classical toxicological studies in animal, bone marrow aspiration are performed and examined in case of circulating blood cell depression. In fact, it is not possible to identify precisely the cell target as well as hematopoietic progenitor as multipotent stem cell in the bone marrow. If abnormal numbers or changes to the circulating blood components are observed, then damage may have already occurred to more primitive cells than that constitute the system. Because the lympho-hematopoietic system comprises hierarchy of cells that begins with an extremely small number of multipotent stem cells and culminates in the production of some 2 million red blood cells and 200,000 white blood cells every second, there is a direct correlation between lympho-hematopoietic damage and the stage at which drug acts. If a drug or any compound acts on one or more different stem cells, ramifications of that effect will be amplified throughout the system. In contrast, if the effect is observed during differentiation, then the effect may be specific for a particular lineage.

As examination of circulating blood parameters cannot be predictive of myelotoxicity because the effect can have already taken place, analysing the effects of xenobiotics on stem cell provide a higher degree of predictability.

2.2. In vitro

2.2.1. Hematopoietic stem cell clonogenic assays have been used to understand the complex system of hematopoiesis for over 40 years

Bradley and Metcalf (1966) and Pluznik and Sachs (1966) introduced the hematopoietic colony-forming assays. These *in vitro* assays detect clonal expansion of multipotent stem cells, progenitor, and precursor cells. They have been validated for clinical aspects.

Differentiation of various cell populations *in vitro* is made possible by the functional ability of these cells to differentiate, in response to growth factors. These assays allow for the detection of an increase or decrease in the frequency of specific hematopoietic progenitor proliferation in response to stimulatory or inhibitory molecules. The culture of cells (2–14 days) permits the proliferation and differentiation of murine or human derived progenitors into colonies containing morphologically identifiable cells (Clarke et al., 2007).

The duration of the assay is in part related to the maturity of the progenitor. Mature progenitors have limited proliferative potential and hence require a relatively short time to achieve maximal colony size (small), whereas the more immature progenitors require longer culture periods to allow the true proliferative potential to be realized, resulting in larger colonies.

In clonogenic assays applied to myelotoxicology, hematopoietic progenitors are seeded in semisolid medium such as agar 0.3%, methylcellulose, or collagen medium in the presence of appropriate cytokines and/or xenobiotics. Cultures are incubated at 37 °C, 5% CO_2, 100% Relative Humidity for 7 days for murine cells and 12–14 days for human cells. Each progenitor gives rise to a colony. Colonies in culture arise from single cells and are thus considered cloned. As progenitors are heterogeneous according to the clone size and the kinetic development, distribution analysis among colonies of different size, in the presence of toxins, allows different expressions of the toxic effect to be distinguished, such as cell destruction, block of mitosis, delay in mitosis, or increase in mitosis number. Dose–effect curves allow determination of the reference dose as $IC_{10,}$ IC_{50} or IC_{90}, the concentrations that inhibit 10%, 50% or 90% of the proliferation, respectively.

There are three types of assays for hematopoietic progenitor clonogenic assays useable in myelotoxicology: CFU-GM assay for Colony Forming Unit Granulocyte and Macrophage, BFU-E assay for Burst Forming Unit Erythroid, and CFU-MK assay for Colony Forming Unit Megakaryocyte.

2.2.1.1. CFU-GM clonogenic assays. Granulopoiesis is the part of hematopoiesis responsible for granulocyte and monocyte production by proliferation and differentiation of specific progenitors, CFU-GM. CFU-GM clonogenic assays have been developed early. Thus their use for toxicological studies began in 1973 (Lind et al., 1973). One can distinguish four steps in their improvement in *in vitro* myelotoxicity.

CFU-GM clonogenic assays were initially utilized in toxicology to confirm the origin in drug-induced agranulocytosis (Ascencao et al., 1984; Barrett et al., 1976; Boogaerts et al., 1984; Irvine et al., 1983; Kelton et al., 1979; Lind et al., 1973; Young and Vincent, 1980). The development of predictive *in vitro* hematotoxicity assays using CFU-GM was developed further. Initially used with human cells, this method has been applied to murine cells in the attempt to perfect a simple, reproducible, and inexpensive test that can be applied to xenobiotics such as pesticides, food, environmental contaminants, and drugs (Parent-Massin and Thouvenot, 1993; Parent-Massin et al., 1996; Lautraite et al., 1995; Gribaldo et al., 1998; Van den Heuvel et al., 1999a,b).

In order to define a test on human cells that could provide an estimate of the tolerable levels and could contribute to the development of therapeutic agents endowed with greater therapeutic activity and less toxicity, in 1995, an ECVAM workshop on the use of *in vitro* systems for the evaluation of hematotoxicity, the participants gave the highest priority to the validation of the granulocyte macrophage colony-forming unit (CFU-GM) assay for predicting acute-onset neutropenia, on the basis of existing evidence related to its usefulness as well as to the similarities between protocols that had been established independently by a number of laboratories (Gribaldo et al., 1996).

Parchment et al. (1998) proposed different models for predicting human MTDs taking into account murine and human IC_{90} values and murine MTD. This model has been tested for food contaminants (Parent-Massin and Parchment, 1998) and validated during the prevalidation phase of the ECVAM program for 10 drugs (Pessina et al., 2000).

The validation phase confirmed the application of the myeloid progenitor colony-forming unit granulocyte macrophage (CFU-GM) assay to predict for the MTD of myelosuppressive compounds. The drug concentration that inhibited the CFU-GM by 90% (IC_{90}) was more predictive of the MTD than the more frequently published IC_{50} value. This very successful study was able to predict