TECHNICAL BULLETIN

Use Of The CFC Assay For Toxicity Testing

Introduction

The hematopoietic colony-forming cell (CFC) assay is a primary cell-based assay that can be used to test effects of environmental toxins, chemotherapeutics and other drug classes on the development of white blood cells, red blood cells and platelets. In the CFC assay, progenitor cells, in response to cytokines and supplements in the culture medium, proliferate and differentiate into mature cell types that can be distinguished morphologically. A change in colony numbers and/or colony morphology of compound-treated cultures compared to a control culture indicates toxicity or inhibition.

The CFC assay is useful for screening compounds for toxicity prior to the initiation of costly clinical trials and as a tool for determining maximum tolerated dose (MTD). Performing the CFC assay with primary cells from different species can assist in determining the animal model most representative of the human condition for a particular class of compound.

A case study performed by STEMCELL Technologies Inc.'s Contract Assay Services evaluated effects of chemotherapeutics and an environmental toxin on hematopoietic progenitor colony-forming units-granulocyte, macrophage (CFU-GM) from human, mouse, rat and canine bone marrow. This case study highlights the effectiveness of the CFC assay in determining which species is comparable to humans with respect to toxicity or inhibition for a particular class of compound.

Case Study: Effects Of Compounds On CFU-GM Progenitors

Compounds Tested

The following compounds were tested for their effect on human, mouse, rat and canine CFU-GM using the CFC assay:

- Topotecan
- Irinotecan
- Camptothecin
- Doxorubicin
- Cisplatin
- 5-Fluorouracil
- Lead Nitrate
- Sunitinib Malate
- Imatinib Mesylate
- Erlotinib HCl

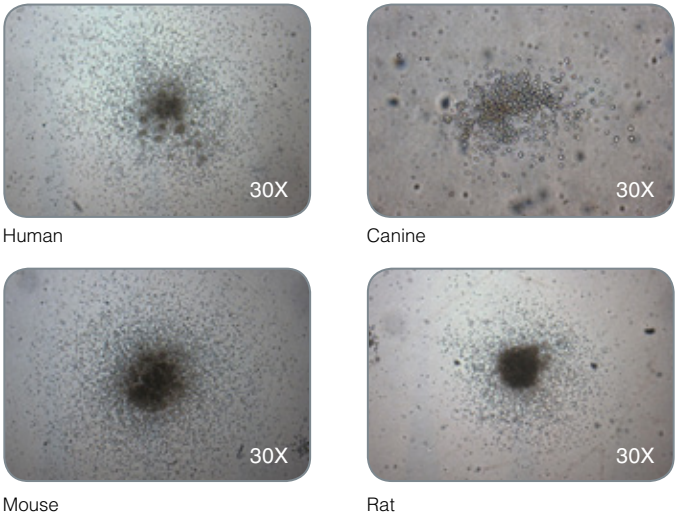
The CFC assay was conducted using cells from the bone marrow of each of the four species and species-specific MethoCult™ methylcellulose-based media.

HUMAN: MethoCult™ GF H84534 (Catalog #84534/84544)
MOUSE: MethoCult™ GF M3534 (Catalog #03534)
RAT: MethoCult™ GF R3774 (Catalog #03774)
CANINE: Customized formulation

Compounds were added directly to the MethoCult™ media and cultures were incubated for the optimal number of days for each species.

Results

FIGURE 1. Representative CFU-GM-derived colonies from each species



Human | Canine | Mouse | Rat

FIGURE 2. Comparison of CFU-GM IC_{50} Values for Topotecan in each species

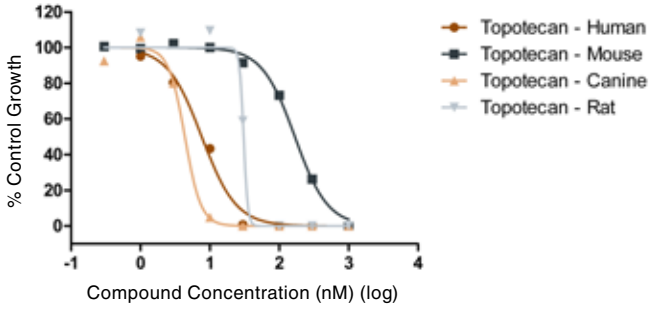


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FIGURE 3. Effect of compounds on human CFU-GM-derived colonies

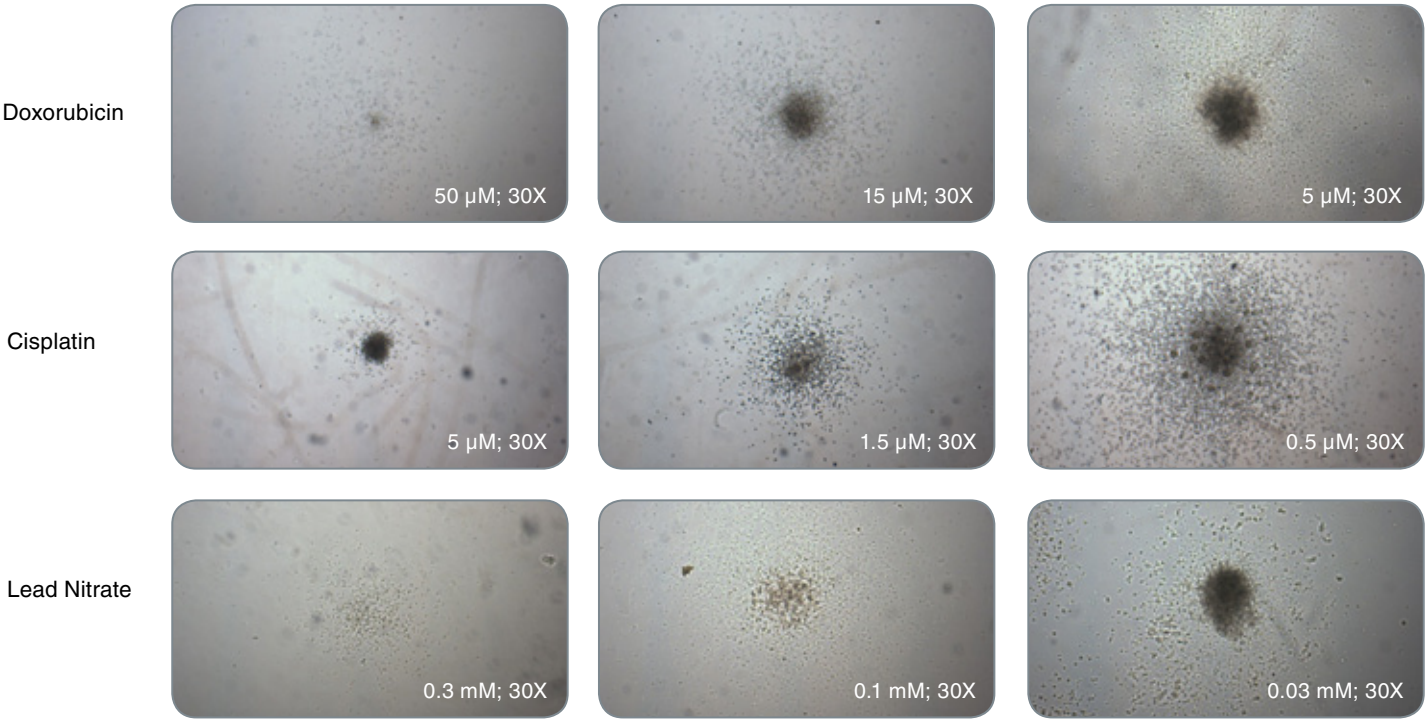

TABLE 1. CFU-GM IC_{50} values for compounds in each species

COMPOUND	HUMAN	MOUSE	CANINE	RAT
Topoisomerases				
Topotecan	**7.69 nM**	168.80 nM	**4.40 nM**	30.96 nM
Irinotecan	**288.10 nM**	>1000 nM	**358.10 nM**	>1000 nM
Camptothecin	**1.03 nM**	6.16 nM	**0.75 nM**	9.16 nM
Anti-Proliferatives				
Doxorubicin	**0.03 µM**	**0.01 µM**	0.002 µM	0.006 µM
Cisplatin	**4.21 µM**	**6.79 µM**	0.97 µM	2.68 µM
5-Fluorouracil	**3.84 µM**	**3.08 µM**	0.23 µM	1.62 µM
Tyrosine Kinase Inhibitors				
Sunitinib	**0.008 µM**	1.10 µM	**0.01 µM**	0.22 µM
Imatinib	**2.16 µM**	>30 µM	**1.99 µM**	>30 µM
Erlotinib	**15.27 µM**	19.39 µM	**10.36 µM**	34.67 µM
Environmental Toxin				
Lead Nitrate	**0.98 mM**	2.05 mM	0.04 mM	**1.20 mM**

Conclusions

- In general, test compounds displayed a dose-dependent toxic effect on myeloid progenitor proliferation
- For most compounds tested, canine myeloid progenitor proliferation was more sensitive than human, mouse or rat myeloid progenitor proliferation
- Different classes of chemotherapeutics as well as an environmental toxin showed species specificity:
  - Topoisomerases and tyrosine kinase inhibitors
    - Human and canine were most similar
  - Anti-proliferatives
    - Human and mouse were most similar
  - Environmental toxin
    - Human and rat were most similar