In vitro testing of angiogenesis by a growth-factor free human based co-culture

Barbara Birk, Nadine Roth, Samuel Woelk, Burkhard Flick, Bennard van Ravenzwaay, Robert Landsiedel

BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany

Angiogenesis, *de novo* formation of blood vessels, is important for (patho-) physiological processes. Chemicals can interact with this biological effect leading to desired (reduced tumor growth) or toxic effects (developmental toxicity). Several external growth factors (GF)-requiring *in vitro* angiogenesis assays have been developed in the past. We developed a human based *in vitro* angiogenesis assay without external GF to screen early anti-angiogenic effects of chemicals.

Human fibroblasts (Cl-huFIB, Inscreenex) were co-cultured with human endothelial cells (HUVEC, PromoCell) in 96-well plate. Cells were treated at Day 4 and 8 with 3 angiogenesis inhibitors with known teratogenic effects (Digoxin (DI), Levamisole (LV), 2-Methoxyestradiol (ME)), two teratogenic substances without angiogenic inhibiting effects (Phenytoin (PH), Methimazole (MT)) and one without anti-angiogenic and teratogenic effects (D-Mannitol (MA)). On Day 14, HUVECs were stained with rabbit-α-Factor VIII related antigen (Zytomed) to quantify the HUVEC tube formation by measurement the tubular length and network branching points (IncuCyte, Sartorius). To distinguish anti-angiogenic effects from cytotoxicity, MTT viability test was performed in parallel.

No external GF was needed to build a reproducible and tubular network. Reproducibility was proven by application of DI in several runs (>n=5). LV, DI, PH and ME showed a lower LOEC (lowest observed effect concentration defined as > 20% inhibitory effects versus vehicle control) for the tube formation than cytotoxicity assuming an anti-angiogenic effect. MT and MA neither showed any inhibitory effect for inhibition of tube formation nor cytotoxicity up to 1000 µM. The results of all test substances in our GF-free assay were in line, except PH, with the results of the method using a co-culture of HUVECs and adipose tissue plus external GF [2]. PH showed anti-angiogenic properties in our assays at >100 µM but was inactive in the assay of Toimelaa et al ([2]). PH is often considered not having anti-angiogenic properties, but Eser et al. [1] could demonstrate this activity in mice.

This GF-free protocol for a human cell-based *in vitro* angiogenesis assay is easy to handle and providing reproducible results in line with literature [1, 2]. Further testing is needed but it could be a useful tool to investigate angiogenesis in the area of toxicology and pharmacology in the future.

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[2] DOI: 10.1016/j.reprotox.2016.11.015