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Letter to the editors

False positive results with MTT assay

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Dear Editors,

The MTT assay has been used extensively since 1983 (Mosmann, 1983), since it represents a simple approach to the evaluation of cell proliferation.

We have performed several tests to study the effect on human endothelial cells (HEC) of selected mediators that have been claimed to induce or inhibit proliferation of these cells, including erythropoietin (EPO) (Carlini et al., 1993), heparin (HE) (Fischer et al., 1990) and cyclosporin A (CyA) (Lau et al., 1989).

Briefly, HEC, isolated from human umbilical vein perfused with 5500 U collagenase (Sigma, St. Louis, MO), were grown in 95% air, 5% CO_2 at 37°C in culture medium Medium 199 (Gibco, Paisley, Scotland) containing: 20% foetal bovine serum (FBS) (Gibco, Paisley, Scotland), penicillin 100 U/ml (Sigma, St. Louis, MO), streptomycin 100 μg/ml (Sigma, St. Louis, MO), amphotericin 0.25 μg/ml (Sigma, St. Louis, MO), 25 mM Hepes (Gibco, Paisley, Scotland), endothelial cell growth factor (Boehringer Mannheim, Germany), HE 20 U/ml (Parke-Davis, Lainate, MI). Cells were grown to confluency and then passaged using a 0.5 mg/ml trypsin-0.25 mmol EDTA solution (Gibco, Paisley, Scotland). Experiments were carried out after the third HEC passage using 96-well plates. Cells were cultured at the concentration of 5000 cells/well for 24 h and for a further 24 h in the presence of EPO (200, 100, 50, 25, 12.5, 6.25, 3.2, 1.5, 0.78, 0.39 U/ml) (Janssen Farmaceutici, Latina), HE (500, 250, 25, 60, 30, 15, 7.5, 3.75 U/ml) (Parke-Davis, Lainate, MI), CyA (5, 2.5, 1.25 mg/ml, 625, 312, 156, 78, 39, 19.5, 9.7, 4.85, 2.4, 1.2 μg/ml, 605 ng/ml) (Sandoz, Wander Pharma, Bern), 10% normal human serum (NHS). MTT and, 2 h later, acid-isopropanol were added according to the Mosmann protocol (Mosmann, 1983). Plates were read at 570 nm.

We observed an apparent dose-dependent proliferative effect induced by HE. The results, expressed as optical densities at different HE con-

Abbreviations: HEC, human endothelial cells; EPO, erythropoietin; HE, heparin; CyA, cyclosporin A; FBS, foetal bovine serum.

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Table 1
Test of interference in MTT assay using a cell-free system

Agent tested	Lysing buffer	20% FBS M199	FBS-free M199	20% FBS, phenol red-free medium	FBS-free, phenol red-free medium	0.9% NaCl solution
5 mg/ml Igs	Isopropanol	0.52	0.52	0.22	0.38	0.38
	SDS	0.16	0.27	0.61	0.97	1.03
25% NHS	Isopropanol	1.68	1.79	1.19	1.27	0.97
	SDS	0.57	0.63	0.73	0.75	0.77
312 U/ml heparin	Isopropanol	0.88	0.20	1.03	0.07	0.09
	SDS	0.05	0.08	0.05	0.03	0.03
5% human albumin	Isopropanol	0.33	0.34	0.13	0.10	0.09
	SDS	0.54	0.50	0.15	0.09	0.09
5% FBS	Isopropanol	0.38	0.31	0.15	0.13	0.11
	SDS	0.21	0.24	0.14	0.10	0.09

FBS, foetal bovine serum; Igs, pooled human immunoglobulins; NHS, normal human serum; CyA, cyclosporin A.
Tests were carried out incubating different media (20% FBS-M199, FBS-free M199, 20% FBS phenol red-free medium, FBS-free phenol red-free medium or 0.9% NaCl solution) with one of the following compounds: 5 mg/ml Igs, 25% NHS, 312 U/ml heparin, 1.25 μg/ml CyA, 5% human albumin, 5% FBS or 500 μg/ml cyclophosphamide. Then MTT was added and isopropanol (according to the Mosmann protocol (Mosmann, 1983)), or SDS (according to Hansen et al., 1989) were used as lysing buffers. Results are presented as optical densities measured at 570 nm.

centrations at time 0 and after 24 h respectively, were: 0.27/0.70 without HE, 0.58/1.60 HE 500 U/ml, 0.43/1.55 HE 250 U/ml, 0.40/1.48 HE 125 U/ml, 0.34/1.37 HE 60 U/ml, 0.28/1.01 HE 30 U/ml, 0.20/0.74 HE 15 U/ml), while CyA and EPO induced no modification of HEC proliferation. Moreover, NHS seemed to induce cell growth.

However, since in control wells, where no HEC had been cultured, high optical densities were also found, we considered the possible non-specific interference of different substances on the MTT. Several authors (Hansen et al., 1989; Nargi and Yang, 1993; Denizot and Lang, 1986; Tada et al., 1986) have already addressed this problem: interference has been reported with serum proteins (Hansen et al., 1989; Denizot and Lang, 1986) from different species (Nargi and Yang, 1993). Hence we performed some tests in a cell-free system, studying the effects of FBS, human serum, HE, CyA, human albumin, human immunoglobulins and cyclophosphamide. Tests were carried out according to the classic protocol (Mosmann, 1983) and using the modification (Hansen et al., 1989) with 20% SDS-dimethyl-formamide solution as lysing agent.

Results are detailed in Table 1. It is interesting to note that when the classic test (Mosmann, 1983) was used, interference was observed with whole serum, FBS, immunoglobulins, human albumin and HE. However, in the latter case, interference was only observed in the presence of FBS. When the protocol with SDS was used (Hansen et al., 1989), this non-specific effect persisted with whole serum, albumin and immunoglobulins. Since no further increase in signal was given by HE and complete culture medium at 24 h in the cell-free system, this interference emphasises the need for a cell-free control at time zero.

In conclusion, the MTT assay is an easy and safe method for measuring cell proliferation, but it must be used with care, since, even with a modified technique (Hansen et al., 1989), false positive results are possible. Substances evaluated for their capacity to modify cell replication rates should always be tested in the absence of cells in order to exclude false positive results.

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