
Suppl. Fig. 1: Dipyridamole does not affect monocytes viability. (A) PBMC were treated with dipyridamole (dip) at the indicated concentrations in RPMI 1630 plus 5% FBS or basal medium plus 0.05 % HSA. After 24 h, the MTT assay was performed as reported in Methods. In no culture condition tested toxicity by dipyridamole treatment was apparent. Data are expressed as mean ± standard deviation (S.D.) (n=16). (B) Phase-contrast images of U937 cells treated with dipyridamole for 24 h and taken before MTT assay. Scale bar = 10 µm, × 20 magnification.
Suppl. Fig. 2: Dipyridamole reduces PMA-stimulated release of MMP-9 at gelatin zymography. PBMC were treated with dipyridamole (dip) for 60 min before PMA stimulation. After 24 h, media were collected and analyzed by gelatin zymography. The image is representative of 3 separate experiments yielding similar results. Densitometric values of gelatinolytic bands derived from 3 independent experiments, each consisting of 4 replicates for each condition, were averaged and reported as units of O.D. expressed as percent of PMA (mean ± SD, n = 3, upper panel). *P<0.05 and **P<0.01 vs PMA alone between groups joined by the horizontal lines. Inset: similar results were obtained using monocytes isolated by the EasySep Human Monocyte Enrichment kit.
Suppl. Fig. 3: Dipyridamole reduces PMA-stimulated release of MMP-9 from U937 cells at gelatin zymography and ELISA. U937 cells were treated with dipyridamole (dip) for 60 min before PMA stimulation. After 24 h, media were collected and analyzed by gelatin zymography (A), and by ELISA (B). The image in (A) is representative of 3 separate experiments yielding similar results. In (B) the original readings were derived from 3 independent experiments, each consisting of 4 replicates for each condition. Values are given as percent of PMA. *P<0.05 and **P<0.01 vs PMA alone between groups.
Suppl. Fig. 4: Dipyridamole reduces MMP-9 release from monocyte-derived macrophages (MDM) at gelatin zymography. Macrophages derived from U937 and THP-1 cells were treated with dipyridamole (dip) at the indicated concentrations. After 24 h, media were collected and analyzed by gelatin zymography. The image is representative of 3 separate experiments yielding similar results. Values of MMP-9 gelatinolytic activity are expressed as arbitrary units of optical density (O.D.) at densitometric analysis (mean ± SD, n = 3). P<0.05 and **P<0.01 between groups joined by the horizontal lines.
Suppl. Fig. 5: Comparative effects of dipyridamole and NAC on TNF-α- and PMA-stimulated release of MMP-9 at gelatin zymography. U937 cells were treated with dipyridamole (dip) or NAC for 60 min before stimulation. After 24 h, media were collected and analyzed by gelatin zymography. The image is representative of 3 separate experiments yielding similar results. Values of MMP-9 gelatinolytic activity are expressed as arbitrary units of optical density (O.D.) at densitometric analysis (mean ± SD, n = 3). *P<0.05 and **P<0.01 between groups joined by the horizontal lines.
Suppl. Fig. 6: Dipyridamole reduces TNF-α- and PMA-stimulated release of t-PA from U937 cells. U937 cells were treated with dipyridamole (dip) for 60 min before TNF-α or PMA stimulation. After 24 h, media were collected and analyzed for PAI-1 (A) and t-PA (B) release by ELISA. The original readings were derived from 3 independent experiments, each consisting of 2 replicates for each condition. Values are given as percent of unstimulated control. *P<0.05 and **P<0.01 vs stimuli alone between groups joined by the horizontal lines.
Suppl. Fig. 7: Dipyridamole reduces the stimulated release of gelatinases, t-PA and PAI-1 from HUVEC. HUVEC were treated with dipyridamole (dip) for 60 min before TNF-α or PMA stimulation. After 24 h, media were collected and analyzed by gelatin zymography (A), and ELISA (B). The image in (A) is representative of 2 separate experiments yielding similar results. Values of MMP-9 gelatinolytic activity are expressed as arbitrary units of optical density (O.D.) at densitometric analysis. In (B) the original readings were derived from 2 independent experiments, each consisting of 2 replicates for each condition. Values are given as percent of unstimulated control. **P<0.01 vs TNF-α alone between groups joined by the horizontal lines.
Suppl. Fig. 8: Dipyridamole, but not 8-bromo-cAMP or db-cGMP, reduces TNF-α-stimulated release of MMP-9 at gelatin zymography and ELISA. U937 cells were treated with dipyridamole (dip), 8-bromo-cAMP or di-butyryl-cGMP (db-cAMP) for 60 min before TNF-α stimulation. After 24 h, media were collected and analyzed by ELISA (A) and by gelatin zymography (B). In (A) the original readings were derived from 3 independent experiments each consisting of 4 replicates for each condition. Values are given as percent of TNF-α. The images in (B) are representative of 3 separate experiments yielding similar results. *P<0.05 and **P<0.01 vs TNF-α alone between groups joined by the horizontal lines.
**Suppl. Fig. 9: Dipyridamole attenuates the activation of AP-1 subunits cJun and FosB.** U937 cells were treated with dipyridamole (dip) for 60 min and then stimulated with TNF-α or PMA for additional 60 min, after which nuclear proteins were extracted and assessed by an ELISA-based TransAM kit. The original readings derived from 2 independent experiments were averaged. Values are given as percent of unstimulated control. *P<0.05 and **P<0.01 vs stimuli alone.
Online Supplement Fig. 10: Dipyridamole does not affect BCL3 expression and phosphorylation. In (A) U937 cells were stimulated with TNF-α or PMA for 0-6 h and then total protein were subjected to Western analysis using an anti-BCL3 antibody. In (B) U937 cells were treated with dipyridamole (dip) for 60 min before TNF-α or PMA stimulation. After 30 min, nuclear and cytosolic proteins were extracted and subjected to Western analysis using an anti-phospho-BCL3 (p-BCL3) antibodies. The image is representative of 3 separate experiments yielding similar results.