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A Comparison of The Effects of Maleimide Derivative and Its Combination With Phorbol-12-Myristate-13-Acetate on Neoplastic Monoblast Cells U-937.

Iryna V Byelinska[1]*, Liudmyla V Garmanchuk[1], Lyudmyla I Ostapchenko[1], Dmytro V Shelest[1], Natalya M Khranovska[2], Yulian M Volovenko[3], and Volodymyr K Rybalchenko[1].

[1]Education and Scientific Centre "Institute of Biology", 3Faculty of Chemistry, Taras Shevchenko National University of Kyiv, 64/13, Volodymyrska Str., Kyiv, Ukraine, 01601
[2]National Cancer Institute, 33/43, Lomonosova Str., Kyiv, Ukraine, 03022

ABSTRACT

Maleimide derivative (MI-1) (1-(4-Cl-benzyl)-3-Cl-4-(CF_3-phenylamino)-1*H*-pyrrole-2,5-dione), an inhibitor of VEGF-R1,2,3(h), PDK1, Src(h), Syk(h), and other protein kinases, *in vivo* decreases the number of colon tumors and monocytes in the blood of rats with colon carcinogenesis. The abovementioned protein kinases are involved in proliferation, differentiation, and functioning of monocytes. The aim of this study was to compare the effects on the U-937 caused by MI-1 alone and its combination with phorbol-12-myristate-13-acetate (PMA) that induced differentiation. The proliferative activity and viability of U-937 were assessed using the MTT and trypan blue exclusion assay. The apoptotic, mitotic, and necrotic cells were evaluated in the cytospin specimens after Pappenheim's staining. The distribution of cell cycle phases was measured by flow cytometry. MI-1 (at 0.008 and 0.016 mM) in combination with 100 nM PMA reduces the number of cells and the viability of U-937 (down to 40% and 62% ($p<0.01$)) as compared to effect of MI-1 alone and PMA alone (down to 35% and 40% ($p<0.01$)) after 24 and 48 h of treatment, respectively, due to inhibition of mitotic activity by 48% ($p=0.003$) and stimulation of apoptosis ($p<0.001$) as compared with MI-1 alone and PMA alone ($p<0.01$, $p=0.018$, respectively). In presence of MI-1, PMA restores the S-phase and reduces G_0/G_1 and G_2/M phases of cells as compared to effect of MI-1 alone, but G_0/G_1 and G_2/M phases of cells are increased comparing to untreated U-937. Thus, the inhibiting effect of MI-1 remains during U-937 differentiation induced by PMA, and MI-1 is a promising agent with antitumor anti proliferative activity.

Keywords: maleimide derivative, phorbol-12-myristate-13-acetate, U-937, mitosis, apoptosis, necrosis

Corresponding author

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INTRODUCTION

The active proliferation of tumor cells is determined by a high activity of signal transduction proteins. High viability of neoplastic monocytic cells is associated with high activity of receptor VEGFR1,2,3 [1], cytoplasmic PDK1 [2], Src (h) [3], Syk (h) [4], and other protein kinases [5]. Maleimide derivative (MI-1, 1-(4-Cl-benzyl)-3-Cl-4-(CF_3-phenylamino)-1H-pyrrole-2,5-dione) was synthesized in silico by the Faculty of Chemistry of Taras Shevchenko National University of Kyiv as a competitive inhibitor of the ATP-binding site of protein kinases [6]. MI-1 in vitro inhibits VEGF-R1,2,3(h), PDK1, FGF-R1(h), EGF-R(h), ZAP70, Syk(h), Src (h), Yes (h), and other protein kinases (proven by ProQuinase GmbH, Germany) as well as colon cancer cells (HCT-116 and SW-6200) [7] and in vivo decreases the number of colon tumors [8], normalizes the increased number of monocytes and platelets in the blood of rats with 1,2-dimethylhydrazine-induced colon carcinogenesis [9], but does not affect the blood cells of rats after chronic exposure [10]. Our previous study demonstrated that MI-1 inhibits proliferation of human neoplastic monoblast cells U-937 via induction of apoptosis and inhibition of mitotic activity of cells due to delay at G_0/G_1 stage [11]. G_0/G_1 arrest by MI-1 was shown in different neoplastic epithelial cells [12].

MI-1 inhibits PDK1, whereas the latter regulates the activity of protein kinase C (PKC) [13]. PKC representa family of serine/threonine protein kinases that directly or indirectly participate in differentiation, cell cycle regulation, and apoptosis [14]. The same isoenzyme can cause opposite effectsin different cells. For instance, the activation of PKCδ induces apoptosis in the acute myeloid leukemia cells, whereas in chronic lymphocytic leukemia cells it exerts antiapoptotic activity ensuring the survival of cells. The activation of PKC in hemopoietic cells directs their differentiation into monocytic cells [15] and is necessary for their functioning [16], which indicates the important role of this protein kinase in the fate of these cells. Since phorbol-12-myristate-13-acetate (PMA) is an agonist of PKC [13, 14], induces the differentiation andinhibits proliferation of neoplastic monoblast U-937 cells [17, 18] similarly to MI-1 [11], which inhibits protein kinases (PDK1, Src(h), etc.), the aim of this study was to compare the effects of the combination of MI-1 and PMA vs. those of MI-1 alone on the morphofunctional state of U-937.

MATERIALS AND METHODS

Cell culture and reagents

U-937 cells were incubated in 96 well plates in the sample volume of 100 µL under normal conditions (5% CO_2, 100% humidity, 37°C) in the culture medium RPMI-1640 (Sigma, USA) in the presence of 10% FBS (Sigma), 2 mM glutamine and 40 µg/mL gentamicin (Biopharma, Ukraine). The cell line was kindly provided by Prof. Filonenko V. V., the Institute of Molecular Biology and Genetics, the National Academy of Sciences of Ukraine). MI-1 at various concentrations and in combination with 100 nM phorbol-12-myristate-13-acetate (PMA) was added to the cell cultures in 100 µL of media after 24 h of cell adaptation with subsequent incubation for 24 and 48 h.

Cell viability and proliferation assay

The viability of cells was assessed using MTT colorimetric test [19]. Briefly, at the end of the culture incubation period, 10 µL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 5 mg/ml) was added to each well. After 4 h of incubation with MTT and subsequent centrifugation of the plate at 1000 g for 20 min, the medium was removed. 100µL of DMSO (Sigma) was added to each well to dissolve the formazan crystals and the optical density was measured at the wavelength of 540 nm.

The number of live and dead cells was calculated in hemocytometer with 0.1% trypan blue exclusion assay.

Quantitative analysis of apoptotic, mitotic, and necrotic cells

The percentage of U-937 cells at the apoptotic, mitotic or necrotic stages was calculated per 1,000 cells using the cytospin prepared specimens stained according to Pappenheim's method [20]. Cytospin specimens were examined using the light microscope (Olympus BX-41, Olympus Europe GmbH, Japan) at the

magnification ×1000, and color micrographs were taken by Olympus C-5050 Zoom (Olympus Europe GmbH, Japan) digital camera at the magnification ×400.

Cell cycle distribution

The proportions of cells in different phases of the cell cycle were measured by flow cytometry with an argon laser (excitation = 488 µm, emission = 585/40 µm) (Becton Dickinson, USA) following propidium iodide staining. The samples were analyzed using Mod Fit LT 3.0 (BDIS, USA) software.

Statistical analysis

The viability of cells was assessed using the curve estimation, quadratic regression model using SPSS 16.0 for Windows. The number of cells (viable, dead, apoptotic, mitotic, and necrotic ones) was normally distributed according to Shapiro-Wilk test result $p>0.05$. The results were statistically processed using Student t-test for comparison of MI-1 and MI-1 in combination with PMA. For comparison of MI-1 in combination with PMA and PMA, ANOVA followed by the post-hoc Hochberg test for number of live and dead cells, assuming homogeneity of variances, and DunnettT3 for apoptotic, mitotic or necrotic cells, not assuming homogeneity of variances, was used. A $p<0.05$ was considered significant. Experimental results are presented in Mean ±SD format.

RESULTS

Influence of MI-1in combination with PMA on the U-937 proliferation and viability

MI-1 modifies the proliferative activity of U-937 resulting in 50% reduction of cell viability and growth at 0.016 mM after 24 h (Fig. 1A) and at 0.008 mM after 48 h (Fig. 1B) of treatment (MTT test result). The antiproliferative activity of MI-1 in combination with 100 nM PMA remains (Fig. 1C, 1D). PMA reduces cell proliferation and induces differentiation.

After 24-h treatment, 0.008 mMMI-1 in combination with 100 nM PMA reduce the proliferation of U-937, which is confirmed by the decreased number of live cells ($0.67\pm0.18\times10^6$/ml) (Fig. 2A), and cell viability (MTT-test result, Fig. 2B) by 40% compared to treating U-937 with corresponding concentration of MI-1 alone ($1.10\pm0.14\times10^6$/ml, $p=0.002$) and by 22% ($p=0.064$) compared to effect of PMA alone ($0.86\pm0.13\times10^6$/ml). The double concentration of MI-1 (0.016 mM) and PMA (100 nM) reduces the proliferation at the same level. The number of live cells ($0.56\pm0.12\times10^6$/ml) and viability are decreased by 40% vs. MI-1 alone ($0.95\pm0.13\times10^6$/ml, $p=0.001$) and by 35% ($p=0.004$) vs. PMA alone. The number of dead cells did not differ across the indicated groups ($0.08\pm0.032\times10^6$/ml after treatment with 0.008 mM MI-1+PMA, $0.08\pm0.028\times10^6$/ml - 0.008 mM MI-1, $0.07\pm0.05\times10^6$/ml - PMA, $0.12\pm0.032\times10^6$/ml - 0.016 mM MI-1+PMA, $0.09\pm0.033\times10^6$/ml - 0.016 mM MI-1, $p\geq0.1$, respectively) (Fig. 2C).

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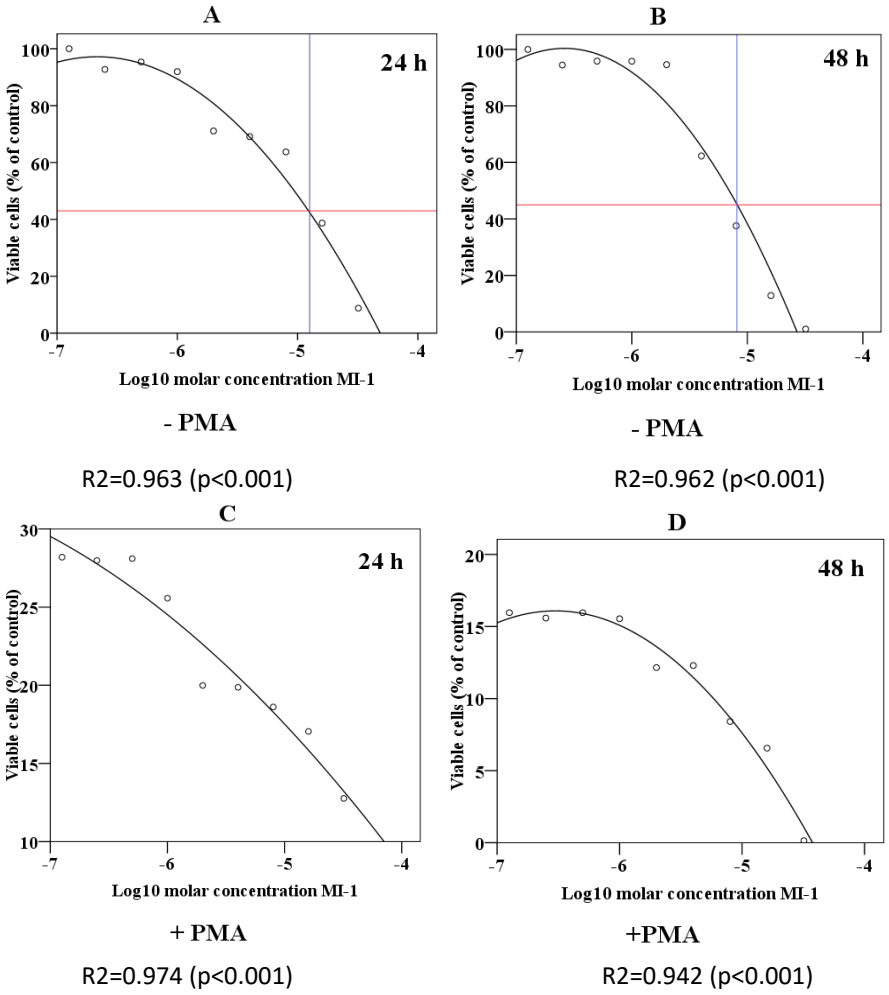

Fig 1 The viability of U-937 treated with MI-1 at the increasing concentration and in combination with 100 nM PMA for 24 and 48 h (MTT test assay), quadratic regression model. (A) U-937 + MI-1, 24 h; (B) U-937 + MI-1, 48 h; (C) U-937 + MI-1 + PMA, 24 h; (D) U-937 + MI-1 + PMA, 48 h; percent of control; the data represent the mean of at least4 independent replicated experiments.

The extension of treatment with 0.008 mM MI-1 and PMA up to 48 h reduces the proliferation. The number of cells decreases by 62% ($0.57\pm0.13\times10^6$/ml) (Fig. 2A) and the viability – by 56% (Fig. 2B) as compared to effect of MI-1 alone ($1.50\pm0.25\times10^6$/ml, $p<0.001$) and by 40% as compared to PMA alone ($0.95\pm0.13\times10^6$/ml, $p<0.001$). However, at 0.016 mM MI-1 and PMA, the number of cells is higher (0.36 ± 0.09, $p<0.028$) as compared to MI-1 alone ($0.20\pm0.09\times10^6$/ml), although it remains significantly lower (by 86%) as compared to untreated control ($2.66\pm0.18\times10^6$/ml) (Fig. 2A), and decreased by 62% ($p<0.001$) as compared to PMA alone. The number of dead cells does not differ between effects of 0.008 mM MI+PMA, 0.008 mM MI and PMA alone ($0.08\pm0.04\times10^6$/ml, $0.12\pm0.04\times10^6$/ml, $0.10\pm0.06\times10^6$/ml, $p=0.1$, respectively) (Fig. 2C), but increased in case of action of 0.016 mM+PMA ($0.32\pm0.09\times10^6$/ml, $p=0.007$) as compared to effect of PMA ($0.10\pm0.06\times10^6$/ml) and does not differ in case of 0.016 mM MI ($0.27\pm0.08\times10^6$/ml).

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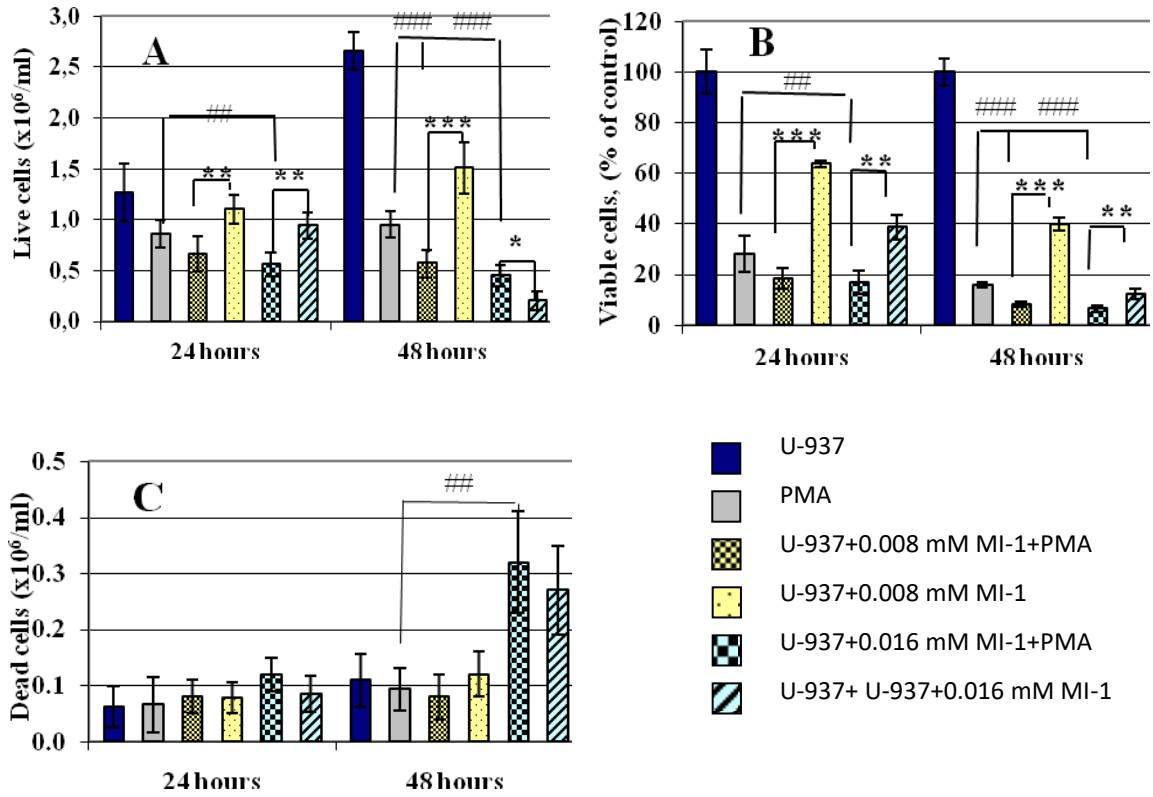

Fig 2 The proliferative activity and viability of U-937 treated with MI-1 at the concentration of 0.008 and 0.016 mM in combination with 100 nM PMA in comparison with MI-1 alone at the corresponding concentration and PMA alone for 24 and 48 h. (A) The number of live U-937 (trypan blue exclusion test); (B) viability of U-937 (MTT- test result); (C) number of dead U-937 (trypan blue positive cells); the data represent the mean of at least 4 independent replicated experiments; *p<0.05, ** p<0.01, *** p<0.001 MI+PMA compared with MI-1 alone at the corresponding concentration (t-test); ## p<0.01, ### p<0.001 MI+PMA compared with PMA (Hochberg test)

Influence of MI-1in combination with PMA on the cell cycle of U-937

The assessment of the cell cycle of U-937, treated with MI-1 at both concentrations in combination with PMA for 48 h, demonstrated the restoration of the S-phase of the cell cycle (30.07±5.44%, p=0.034; 35.57±3.86%, p=0.003) with the consequent reduction of proliferative rest cells in G_0/G_1 (62.29±4.98%, p=0.035; 60.86±3.67%, p=0.007) (Fig. 3A) and in proliferative G_2/M phases (7.64±0.46%, p=0.028; 3.57±0.19%, p<0.001) (Fig. 3B) as compared to U-937 treated with MI-1 in the relevant concentration (14.74±6.43%, 15.70±3.49%; 76.41±6.01%, 76.35±3.67%; 8.85±0.42%, 7.86±0.32%, respectively).The restoration of S-phase of cells, probably, prolongs U-937 lifetime, which is confirmed by a higher number of cells after 48 h of treatment with 0.016 mM MI-1 and PMA as compared to MI-1 alone (Fig. 2A). At the same time, the number of U-937 increases in G_0/G_1 phase and decreases in S-phase after the treatment with MI-1 in combination with PMA and MI-1 alone comparing to untreated U-937 (38.55±3.05%, 56.47±3.30% respectively) (Fig. 3A). Percent of U-937 cells in G_0/G_1 and S phases is not different after the treatment with MI-1 at both concentration in combination with PMA and PMA alone (61.94±3.10%, p=0.999, p=0.969; 37.47±3.06%, p=0.281, p=0.874 respectively), but increases in G_2/M phase (PMA - 0.59±0.05%, p=0.003, p=0.002). As the combined action of MI-1 and PMA decreases the number of viable U-937 cells (Fig. 2A), regardless of the restoration of S- and G_2/M phases of cells, the studies of the morphofunctional state of cells were conducted.

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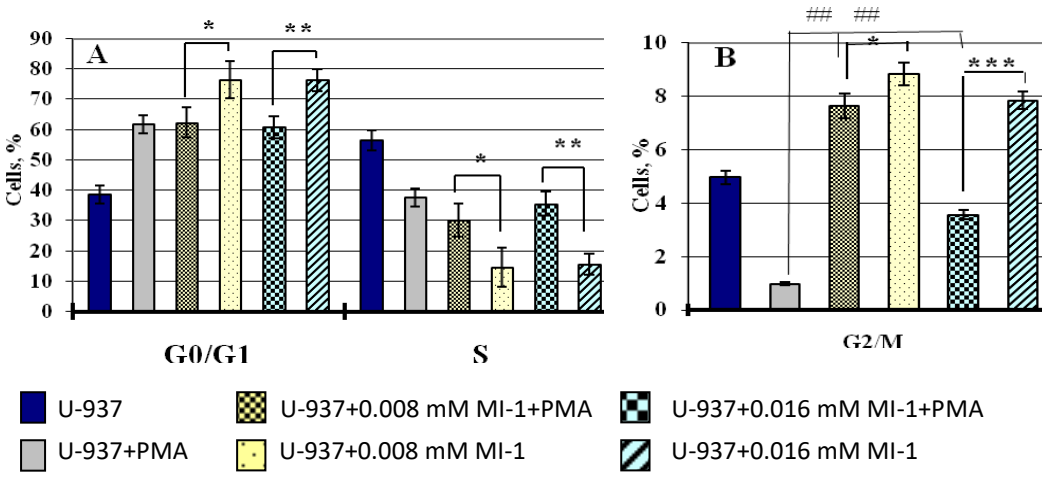

Fig 3 The cell cycle distribution of U-937 treated with MI-1 at the concentration of 0.008 and 0.016 mM in combination with 100 nM PMA in comparison with MI-1 alone at the corresponding concentration and PMA alone for 24 and 48 h. (A)Percent of U-937 in G_0/G_1 and S phases; (B) percent of U-937 in G_2/M phase; the data represent the mean of 3 independent replicated experiments (n=3);* p<0.05, ** p<0.01, *** p<0.001 MI+PMA compared with MI-1 alone at the corresponding concentration (t-test); ## p<0.01 MI+PMA compared with PMA (DunnetT3 test)

Influence of MI-1in combination with PMA on apoptosis, mitosis and necrosis of U-937

The 24-h treatment of U-937 with the combination of MI-1 at the concentration of 0.008 mM and PMA does not change the number of apoptotic cells (2.57 ± 0.32%) in comparison with MI-1 alone (3.27±0.95%, p=0.293) and PMA alone (2.03±0.31%, p=0.245) (Fig.4A, 5A-D); whereas at the concentration of 0.016 mM in combination with PMA the above mentioned number increases (17.17±2.52%) compared with MI-1 (10.40±2.03%, p=0.022) and PMA (2.03±0.31%, p=0.018) (Fig.4A, 5E, 5F). Apoptotic U-937 cells are presented in the photomicrographs (Fig. 5a). The rise of apoptotic cells is accompanied by the reduction in the number of mitotic cells by 20% at 0.008 mM MI-1 with PMA (1.90±0.10%) and by 40% at the 0.016 mM MI-1 with PMA (1.60±0.10%) as compared to MI-1 alone at the corresponding concentration (2.40±0.36%, p=0.082; 2.70±0.26%, p=0.003 respectively) and PMA (2.50±0.10%, p=0.005, p=0.001 respectively) after 24 h of exposure (Fig. 4B, 5b).

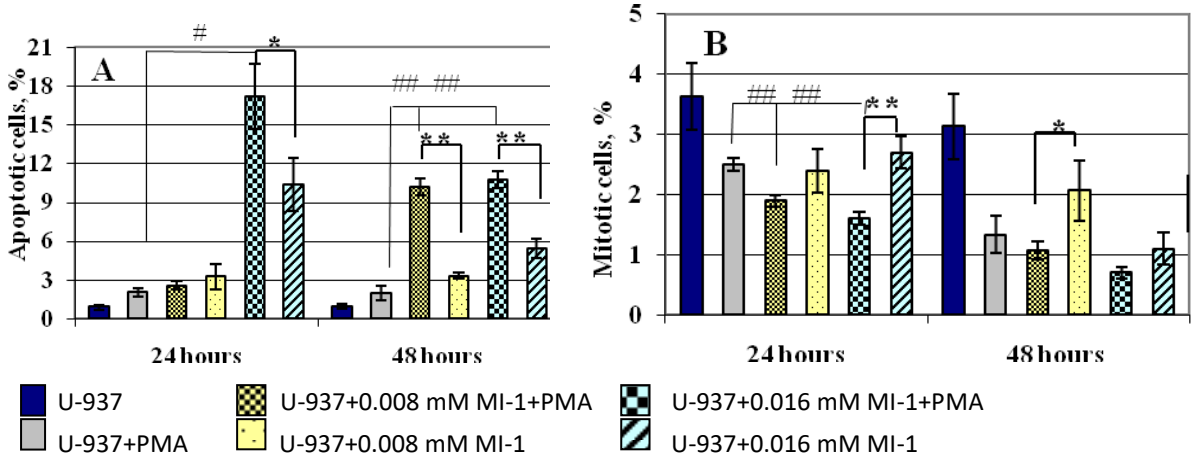

Fig 4 The content of U-937 cells in apoptosis and mitosis treated with MI-1 at the concentrations of 0.008 and 0.016 mM in combination with 100 nM PMA and MI-1 alone at the corresponding concentration and PMA alone for 24 and 48 h. (A) apoptotic cells, (B) mitotic cells; the data represent the mean of 3 independent replicated experiments (n=3);* p<0.05; ** p<0.01 MI+PMA compared with MI-1 alone at the corresponding concentration (t-test); #p<0.05, ## p<0.01 MI+PMA compared with PMA (DunnetT3 test)

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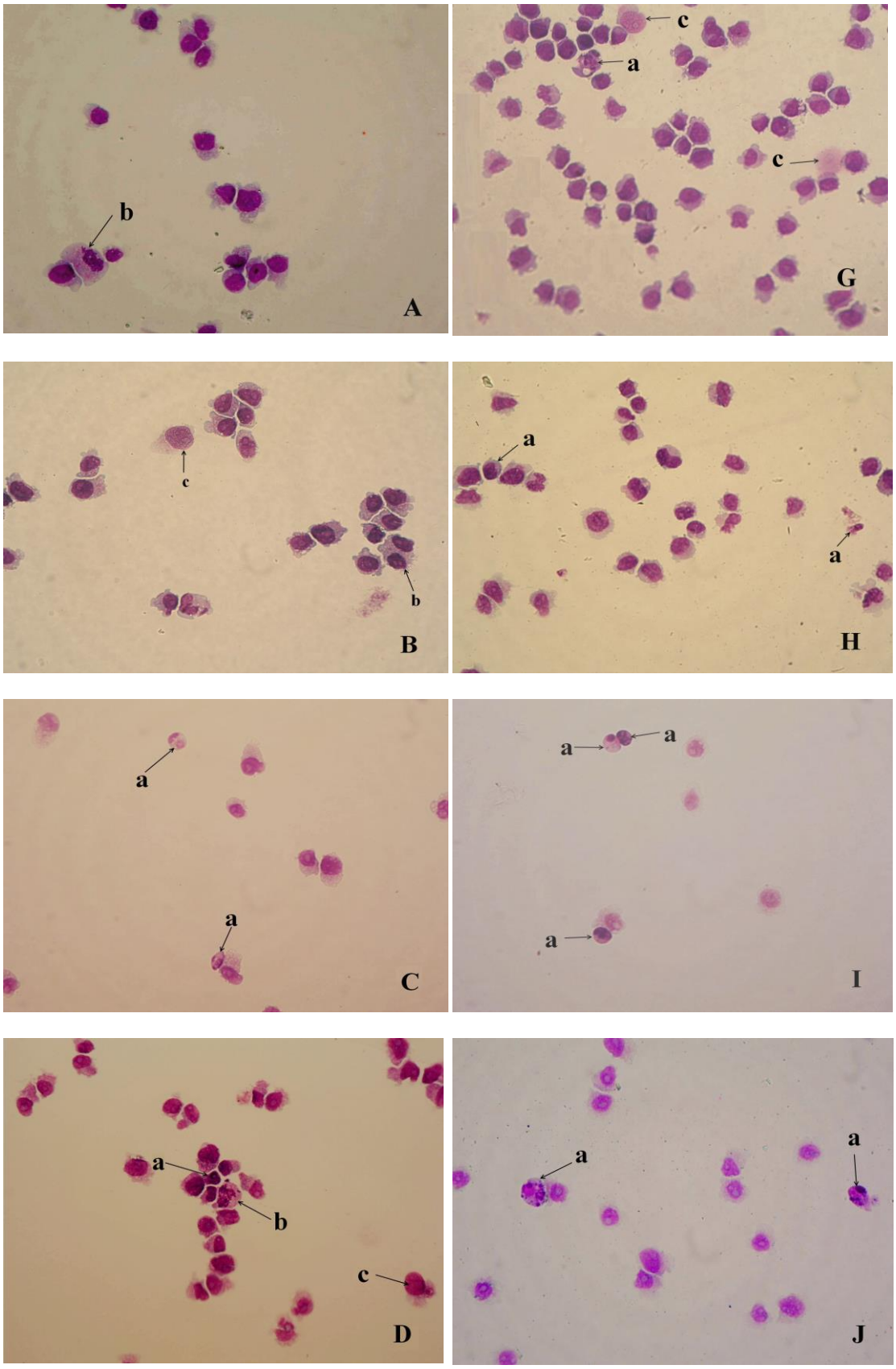

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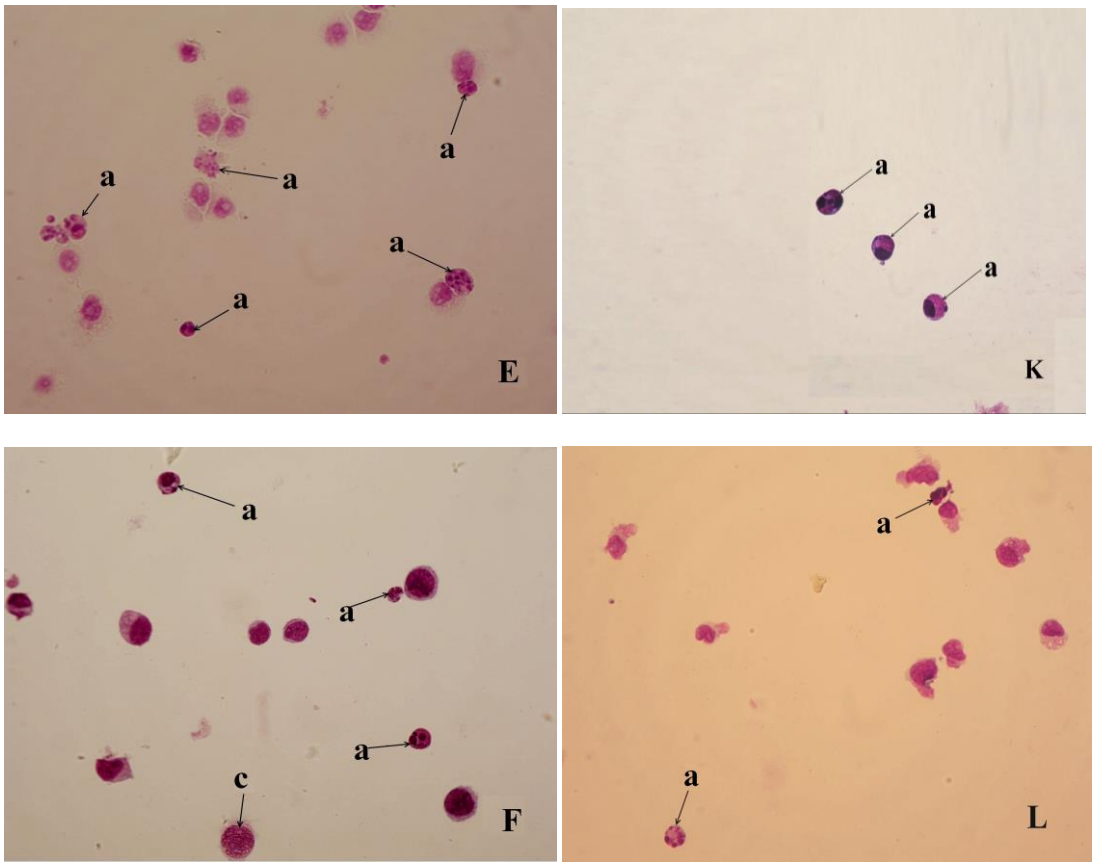

Fig 5 Photomicrographs of cytospin specimens of U-937 cells after 24 and 48 h of treatment with MI-1 at the 0.008 and 0.016 mM in combination with 100 nM PMA and MI-1 alone at the corresponding concentration and PMA alone, Pappenheim staining, ×400; (A) U-937, 24 h; (B) U-937+PMA, 24 h; (C) U-937+0.008 mM MI-1+PMA, 24 h; (D) U-937+0.008 mM MI-1, 24 h; (E) U-937+0.016 mM MI-1+PMA, 24 h; (F) U-937+0.016mM MI-1, 24 h; (G) U-937, 48 h; (H) U-937+PMA, 24 h; (I) U-937+0.008 mM MI-1+PMA, 48 h; (J) U-937+0.008 mM MI-1, 48 h; (R) U-937+0.016 mM MI-1+PMA, 48 h; (L) U-937+0.016 mM MI-1, 48 h; (a) apoptosis, (b) mitosis, (c) necrosis

The extension of the U-937 exposure to MI-1 at both concentrations and in combination with PMA for 48 h results in 3- and 2-fold increase in the number of apoptotic cells (10.17±0.15%, 10.73±0.64% respectively) comparing to MI-1 at the corresponding concentration (3.33±0.25%, $p<0.001$; 5.47±0.76%, $p=0.001$ respectively) and 5-fold as compared to PMA (2.00±0.53%, $p\leq0.001$) (Fig. 4A, 5G-L). The mitotic activity was reduced by 48% at 0.008 mM MI-1 with PMA (1.07±0.15%) and by 36% at 0.016 mM of MI-1 and PMA (0.70±0.10%) compared with MI-1 alone at the corresponding concentration (2.07±0.50%, $p=0.030$; 1.10±0.26%, $p=0.070$ respectively) and did not differ from the effect of PMA (1.33±0.31%) (Fig. 4B).

As the cells can die due to both apoptosis (Fig. 5a) and necrosis (Fig. 5c), the number of cells at the stage of necrotic death was assessed. The amount of necrotic cells after the treatment with MI-1 at the 0.008 mM in combination with PMA decreases after both 24 h (2.63±0.15%) and 48 h (1.10±0.10%) treatments compared to MI-1 alone (5.00±0.66, $p=0.004$; 1.90±0.36%,$p=0.021$, respectively) and PMA (5.10±0.36,$p=0.001$) (Fig. 6). The double concentration of MI-1 in combination with PMA causes an increase in the necrotic cell death after 24 h treatment (4.27±0.31%) compared to MI-1 (2.13±1.07%, $p=0.029$) and shows no differences vs. PMA ($p=0.060$). After 48 h treatment no differences (2.17±0.49%) were observed as compared to effects of MI-1 (2.17±0.40%, $p=1.00$) and PMA (0.87±0.31%, $p=0.060$). It suggests that the absence of the increase in the number of necrotic cells after the 48 h treatment with 0.016 mM MI-1 and PMA is related to the active cytolysis of the cells and impossibility to isolate them in cytospin specimens, which is confirmed by a considerable number of necrotic cells in the suspension after trypan blue staining (Fig. 2C). It is also noteworthy that a higher concentration of MI-1 has an increasing modulating impact on PKC activity and its effects.

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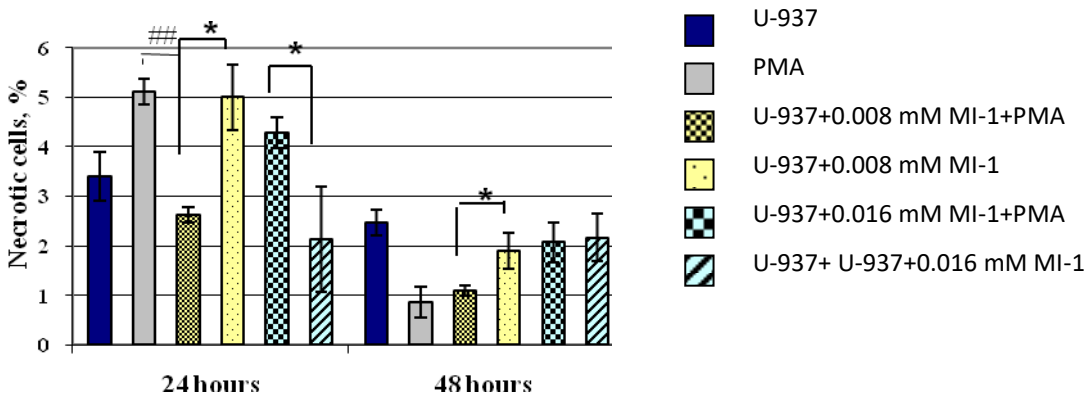

Fig 6 The content of U-937 cells in necrosis treated with MI-1 at the 0.008 and 0.016 mM in combination with 100 nM PMA and MI-1 alone at the corresponding concentration and PMA alone for 24 and 48 h; the data represent the mean of 3 independent replicated experiments (n=3); * $p<0.05$ MI+PMA compared with MI-1 only at the corresponding concentration (t-test); ##$p<0.01$MI+PMA compared with PMA (DunnetT3 test)

Thus, MI-1 in combination with the agonist of protein kinase C, PMA, decreases the proliferation and viability of U-937 due to elevation of its apoptosis and reduction of mitotic activity in comparison with MI-1 and PMA alone. However, PMA in the presence of MI-1 restores the S-phase of the cell cycle with the corresponding reduction of the cells in G_0/G_1 and G_2/M phases and, probably, increases both the lifetime and the number of the cells after 48 h exposure to MI-1 at 0.016 mM with PMA. At the same time, the number of U-937 increases in G_0/G_1 and G_2/M phases and decreases in S-phase, which is accompanied by a decrease in a number and viability of the cells treated with MI-1 in combination with PMA and by MI-1 alone as compared to untreated U-937.

DISCUSSION

Suppression the proliferative activity of neoplastic hematopoietic cells by inhibiting the activity of various kinases is successfully used in oncology in the last decade [21-27]. It was shown in the previous studies that the administration of protein kinase inhibitor MI-1 in the doses of 0.027 and 2.7 mg/kg, which correspond to the concentration of $\sim 10^{-6}$ and $\sim 10^{-4}$M in the blood, decreases the number and size of colon tumors in 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in vivo [8]. After the chronic exposure, MI-1 in the indicated doses does not affect the morpho functional state and count of red blood cells, leukocytes, and platelets in healthy rats [10]. Since MI-1 does not have any negative effect on hematopoiesis and other tissues [8, 28, 29], there are no limitations for the use of this compound as a potential antitumor drug. At the same time, MI-1 at the micro molar concentration reduces the proliferative and mitotic activity, causes delays in the G_0/G_1 phase of the cell cycle and induces the apoptosis of neo plastic mono blast U-937 cells [11]. The activation of PKC in the normal hematopoietic cells and U-937 induces their differentiation into mono cytes and macrophages [15-17], whereas high doses of activators (PMA>100 nM) induce apoptosis, mediated activation, and mitochondrial translocation of PKCβII [17]. The activation of PKCδ also results in apoptosis of leukemic cells [30]. On the contrary, high activity of PKCα and PKCc/n in myeloid leukemic cells facilitates their high proliferative activity and survival after the effect of cytotoxic preparations [2, 31]. The abovementioned demonstrates the heterogeneity of PKC and its corresponding different roles in the fate of cells: proliferation, viability, and apoptosis. Since both protein kinases inhibitor MI-1 [11] and high-dose PKC activator PMA [17, 18] induce U-937 apoptosis, and PDK1, Src, and other kinases regulate PKC by inhibiting MI-1 [14], the study of their combined action on U-937 was warranted. This study demonstrated that the combined effect of MI-1 and PMA enhances apoptosis and inhibits mitotic and, thus, proliferative activity of U-937 compared to MI-1 alone, regardless of the PMA-induced restoration of the S-phase cell cycle of U-937 with consequent reduction of cells in G_0/G_1 (proliferative rest) initiated by MI-1. Thus, the inhibiting effect of MI-1 remains during U-937 differentiation induced by PMA. Therefore, it is possible to conclude that MI-1 induces the inhibition of proliferation and the activation of apoptosis not only via PKC, since the abovementioned effects of MI-1 and PMA are combined, which is confirmed by the comparison of our data on the combined effect of MI-1 with

PMA against PMA alone. It should be noted that MI-1 initiates cell cycle arrest in G_0/G_1 phase mainly, and in G_2/M – partially. Under conditions of the combined effect of MI-1 and PMA, the G_0/G_1 arrest is preserved, but it is less expressed compared to the effect of MI-1, as PMA restores the S-phase and abolishes the G_2/M arrest. PMA is well known to induce G_0/G_1 arrest in U-937 [18], which is in good agreement with the results of this study, and G_2/M cell cycle arrest as was previously shown on lung carcinoma cells [32], but when these cells were previously synchronized in G_1 phase PMA inhibits the progression into S phase, thus PKCs trigger distinctive responses when activated in different phases of the cell cycle [33]. Also, the results of this study demonstrated the modulating effect of PMA on the intensity of cell necrosis. It was shown that PMA with 0.008 mM MI-1 decreases cell necrosis after both 24-h and 48 h treatments, whereas in combination with 0.016 mM of MI-1 PMA causes an increase in cell necrosis after 24 h treatment as compared to MI-1 only. At the same time, the action of a double concentration of MI-1 results in the increase in the number of necrotic cells, which may be related to the summation of the destructive effect on cells via modulation of PMA activity by decreasing of its effect. The protective effect of PMA on the TNF-induced necrotic death of cells was also shown using different lines of cells [34].

CONCLUSION

MI-1 in combination with PMA enhances the inhibition of proliferation of U-937 compared with MI-1 alone and PMA alone due to the elevation of apoptosis and reduction of mitotic activity. PMA in the presence of MI-1 restores the S-phase and reduces G_0/G_1 and G_2/M phases of cells as compared to MI-1 alone. At the same time, the number of U-937 increases in G_0/G_1 and G_2/M phases and decreases in S-phase treated with MI-1 in combination with PMA and MI-1 alone as compared to untreated U-937. Thus, the combined effect of MI-1 and PMA results in the decrease in the number of viable U-937 cells as compared to effects of MI-1 alone and PMA alone and to untreated U-937. Antiproliferative effect of MI-1 remains during U-937 differentiation induced by PMA. MI-1 is a promising agent with antitumor antiproliferative activity.

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