Original article

Effect of dimethyl sulfoxide on mouse embryo fibroblasts: inhibition of plasminogen activator inhibitor deposition and interference with early events of serum-stimulated growth

Thandavarayan NAGASHUNMUGAM, Shankara SRINIVAS and Govindaswamy SHANMUGAM*

Cancer Biology Division, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

Quiescent and serum-stimulated cultures of Swiss mouse embryo fibroblasts (MEF) showed alterations in cell morphology including an enlargement in size upon treatment with 2% dimethyl sulfoxide (DMSO). Treatment of MEF and monkey kidney epithelial cells (MK2) with 2% DMSO at the early periods of serum-stimulated growth inhibited RNA, protein and DNA synthesis. DMSO treatment of cells at late stages of serum-stimulated growth (MEF after 1 hr and MK2 cells after 3 hr of stimulation) had little effect on DNA and protein synthesis although cell enlargement occurred in these cells. When the [^{35}S]methionine labelled proteins of the control and the DMSO treated cells were analysed by high resolution polyacrylamide gel electrophoresis, no apparent difference was observed in the pattern of intracellular proteins of these cells. In contrast, the extracellular levels of two serum-induced secreted proteins of MEF (M_r 48000 and 26000) were dramatically reduced by DMSO treatment. The DMSO sensitive 48 kDa protein was found to be the major component of the extracellular matrix, while the 26 kDa protein was not. The 48 kDa protein was identified as plasminogen activator inhibitor (PAI-1). Densitometric quantitation showed a gradual accumulation of this protein in the matrix of serum-stimulated cells. The deposition of this protein in the matrix was inhibited by DMSO. Flow-cytometric quantitation of indirect immunofluorescence indicated higher intracellular levels of the 48 kDa protein in fetal calf serum (FCS)+DMSO treated cells, suggesting that the low level of this protein in the medium of DMSO treated cells is probably due to lack of transport of this protein from the cells into the medium.

DMSO — cell proliferation — PAI-1 secretion — morphological changes

INTRODUCTION

Treatment of cultured cells with dimethyl sulfoxide (DMSO) resulted in a variety of effects. DMSO is a potent inducer of cell differentiation [1] which affects cell morphology [2–5]. The growth of some solid tumors has been shown to be inhibited by DMSO [2]. The polar solvent has been shown to increase the adhesiveness of low metastatic clones of 3LL carcinoma [6]. These effects were accompanied by reduced tumorigenecity and reversal of the malignant phenotype of cells to normal phenotype [7–11]. DMSO treatment enchanced albumin production in transformed hepatocytes [9], and decreased the binding of EGF to its receptor [12]. The production of plasminogen activator in human carcinoma was inhibited by DMSO [5]. Addition of DMSO selectively enhanced the incorporation of [^{3}H]thymidine into cultured Novikoff hepatoma cells [13] but retarded the growth of murine erythroleukemia cells and led to the differentiation process [14]. Rubin and Earp [15] have shown that

DMSO selectively stimulated phosphorylation of tyrosine residues of the rat liver EGF receptor.

To further understand the mode of action of DMSO, we have studied its effect on cultured mouse embryo fibroblasts. We have specifically studied its effect during mitogenic stimulation of quiescent cells. The results presented here indicate that DMSO treatment changes the morphology of the cells and interferes with crucial events of mitogenic stimulation. The extracellular levels of two serum-induced proteins of M_r 48000 and 26000 were affected by DMSO treatment. Under these conditions, the serum-induced DNA synthesis was also inhibited suggesting the possible involvement of the DMSO sensitive 48 kDa and 26 kDa proteins in the transfer of a mitogenic signal.

MATERIALS AND METHODS

Primary cultures of Swiss mouse embryo fibroblasts were maintained in Eagle's minimum essential medium (MEM, Flow Labs, UK) containing 10% bovine serum (Sera Labs, UK) and 50 μg/ml gentamycin. Cells from the fourth passage onwards (secondary cultures) were used for experiments. Subconfluent monolayers were synchronized at quiescence by maintaining in 0.5% serum

---

containing medium for 72 hr. Cells were stimulated from quiescence by the addition of medium containing 20% fetal calf serum. Cells were treated with 2% DMSO unless otherwise mentioned.

Radiolabelling of intracellular and secreted proteins

Quiescent and stimulated cultures, grown in the presence and absence of DMSO, were labelled with 25 μCi of [^{35}S]methionine (Amersham, specific activity 1390 Ci/Mmol) at 37°C in Hank's balanced salt solution for 30 min. The radioactivity was chased for 30 min by incubating the cells in serum-free medium. The secreted proteins present in the chase-medium were analysed as mentioned earlier [16]. Cells were scraped in phosphate buffered saline (PBS) and lysed in the electrophoresis sample buffer. All samples were electrophoresed in 5–18% polyacrylamide gradient gels containing sodium dodecyl sulfate (SDS).

The extracellular matrix (ECM) proteins were isolated as described by Laiho *et al.* [17]. Immunopreciptation was carried out as described earlier [18].

Analysis of nucleic acids

DNA synthesis was monitored by labelling the monolayers (25 cm²) with 10 μCi of [^{3}H]thymidine for 30 min. At the end of labelling, cells were scraped, suspended in PBS and an equal volume of 20% trichloroacetic acid was added. The precipitate was collected under vacuum suction and the radioactivity retained in the filter was quantitated in a Packard liquid scintillation counter. For monitoring RNA synthesis, 25 cm² monolayers were labelled with 10 μCi of [^{3}H]uridine for 30 min and the incorporated radioactivity was determined as described above.

Flow cytometry

Cell size was determined by flow cytometry using a fluorescence activated cell (FAC) scanner (Becton and Dickinson). Cells were trypsinised, washed with PBS and fixed in 75% ethanol for 1–6 hr. Aliquots containing 2 × 10⁶ cells were used for cell-size analysis and 10000 cells were sized with forward and side scattering.

Indirect immunofluorescence

Cells were collected in cold PBS, pelleted, fixed by the addition of 3.7% formaldehyde in PBS and vortexed to avoid cell aggregation. Fixed cells were permeabilised by 0.1% Triton X-100 treatment for 10 min, pelleted and then treated with rabbit antibody to plasminogen activator inhibitor (PAI-1) for 30 min at room temperature. Excess antibody was removed by extensive washing of the cells with PBS before suspending them in FITC conjugated goat anti-rabbit IgG. Excess conjugate was removed by washing thrice with PBS and the fluorescence was monitored in the FAC scanner by gated scatter analysis. The percentage of positive cells in each histogram was taken from the right side of the gate.

RESULTS

Effect of DMSO on cell morphology

Photomicrographs of quiescent, serum-stimulated and DMSO treated mouse embryo fibroblasts are shown in Figure 1. Quiescent cells had very small nuclei. However,

when the quiescent cells were stimulated with 20% FCS, the cells showed enlargement in size and had large prominent nuclei. Treatment of quiescent cells with serum and DMSO led to further enlargement in size. The cytoplasmic to nuclear volume ratio was altered in DMSO treated cells as compared to the untreated cells. The DMSO treated cells appeared more flattened and extended lengthwise. The changes in cell sizes in quiescent, FCS and FCS + DMSO treated cells were confirmed by flow cytometry. The results presented in Figure 1 D and E show the cell size increase in the following order: quiescent cells < serum-stimulated cells < quiescent cells treated with DMSO < serum-stimulated cells treated with DMSO. The additive effect of serum and DMSO in increasing the cell-size is explicit in these experiments (Fig. 1 E).

Effect of DMSO on secreted proteins

Quiescent cultures of mouse embryo fibroblasts were stimulated with medium containing 20% FCS and grown in the presence and absence of DMSO. After 6 hr of DMSO treatment, cells were pulse-labeled with [^{35}S]methionine. Following 60 min chase incubation, the secreted proteins were collected and analysed by SDS–PAGE. The electrophoretic profile shows a drastic reduction in the level of two secreted proteins of M_r 48000 and 26000 in the medium of cells that were treated with FCS + DMSO (Fig. 2). These proteins were absent in the conditioned medium of quiescent cells but were present in the medium of serum-stimulated cells. N-chlorosuccinamide cleaved peptide mapping of the 48, 45, 40, and the 26 kDa secreted proteins and actin did not show any relationship to each other (results not shown).

Effect of DMSO on matrix proteins

The extracellular matrix proteins of DMSO treated and untreated cells (both quiescent and serum-stimulated) were analysed by SDS–PAGE. The 48 kDa secreted protein was found to be the major component of the extracellular matrix while the 26 kDa secreted protein was a non-matrix protein (Fig. 3, lanes A–D). DMSO inhibited the deposition of the 48 kDa protein in the ECM of both quiescent (lane B) and serum-stimulated (lane D) cells. Densitometric quantitation showed 82% reduction in the level of the 48 kDa protein in the matrix of quiescent cells treated with DMSO for 6 hr in comparision to its level in the matrix of untreated quiescent cells. A 24% reduction in the level of this protein in the matrix of FCS + DMSO treated cells (6 hr treatment) was observed in comparision to its level in cells treated with FCS alone. The 48 kDa protein was barely visible in the electrophoretic profile of the medium proteins of quiescent cells (Fig. 2E) while it is prominent in the matrix protein profile of these cells (Fig. 3A).

The time course of deposition of the 48 kDa protein in the matrix was followed in serum-stimulated cells in the presence and absence of DMSO and the results are presented in Figure 4. Two hours after stimulation of quiescent cells with FCS, a 10% reduction in the deposition of the 48 kDa protein in the matrix was observed in the presence of DMSO; the deposition of this protein progressively declined to 75% at the end of 10 hr of DMSO treatment of serum-stimulated cells. The reduced levels of the 48 kDa protein in the medium of the DMSO treated
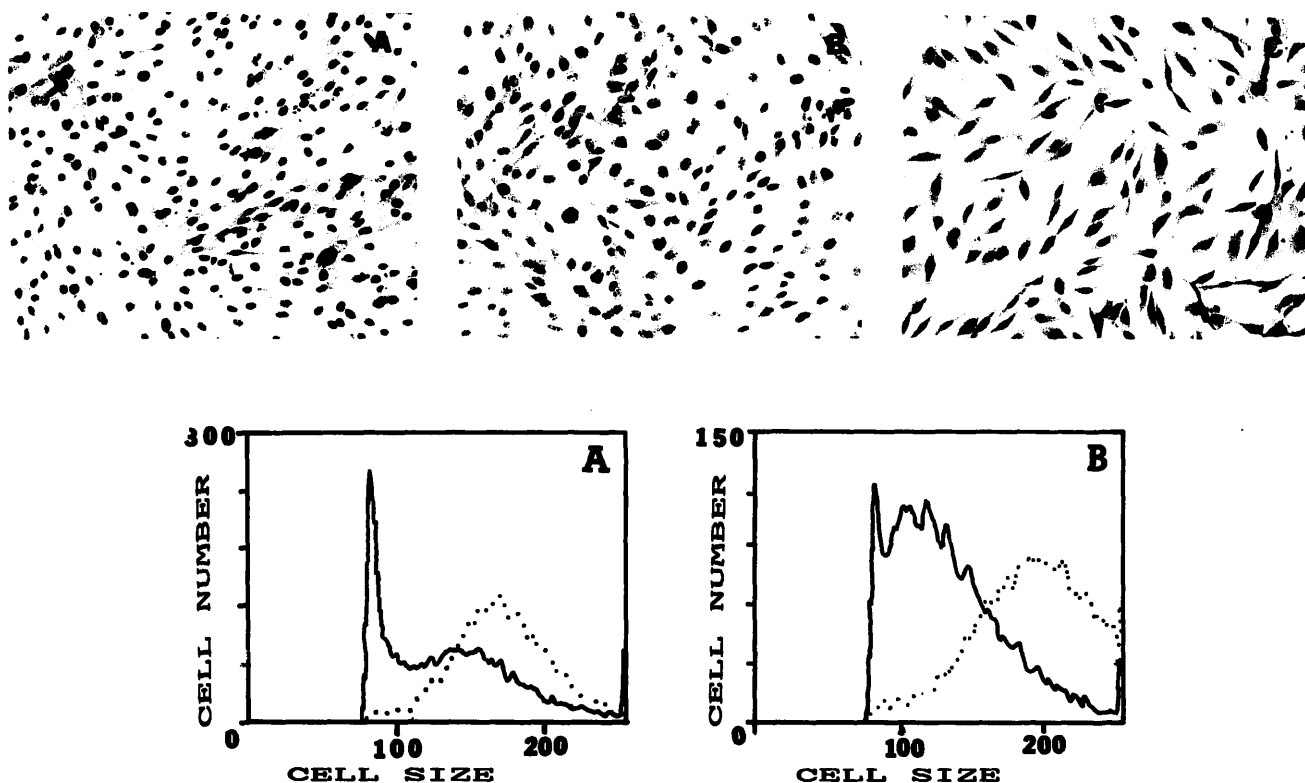
FIGURE 1. — Morphology and cell-size analysis of MEF treated with DMSO. Cells were synchronized at quiescence by growing them in medium containing 0.5% serum for 72 hr. Quiescent cells were stimulated to grow by the addition of medium containing 20% FCS and treated with 2% DMSO. After 6 hr of DMSO treatment, cells were stained with Giemsa and photographed. A, quiescent cells; B, FCS stimulated cells; C, FCS (20%) + DMSO (2%) treated cells. The graphs depict flow cytometric analysis of cell-size of the quiescent (D) and FCS stimulated (E) cells. In D and E, the dotted lines represent the cell-size distribution of the DMSO treated cells. In each case 10000 cells were analysed.

cells may be due to inhibition of transport of this protein into the medium. This was indeed is the case since the intracellular level of this protein was more in serum-stimulated cells that were treated with DMSO than that was observed in cells treated with serum·alone (Table I). There was no significant difference in the PAI-I specific fluorescence of resting cells treated with DMSO and the untreated cells.

Identity of the 48 kDa protein

Since plasminogen activator inhibitor (M_r 48000) was reported to be a major matrix component [17] and our studies showed the 48 kDa protein as the major matrix protein, antibodies to PAI-1 were used to identify this protein. Immunoprecipitation studies showed that the 48 kDa protein was precipitated by antibodies to plasminogen activator inhibitor (PAI-1) suggesting that the 48 kDa secreted protein is related to PAI (Fig. 3, lanes F and G).

TABLE I. — Quantitation of PAI-1 by indirect immunofluorescence.

Cells	Percent fluorescence[a]
FCS stimulated cells reacted with normal serum	14.5
Quiescent cells reacted with PAI-1 antibody	42.2
FCS stimulated cells reacted with PAI-1 antibody	27.2
FCS + DMSO treated cells reacted with PAI-1 antibody	60.7

[a]Fluorescence was quantitated by flow cytometry as described in Materials and Methods. In indirect immunofluorescenc measurements, normal rabbit serum treated cells were used as control to determine non-specific and background fluorescence.
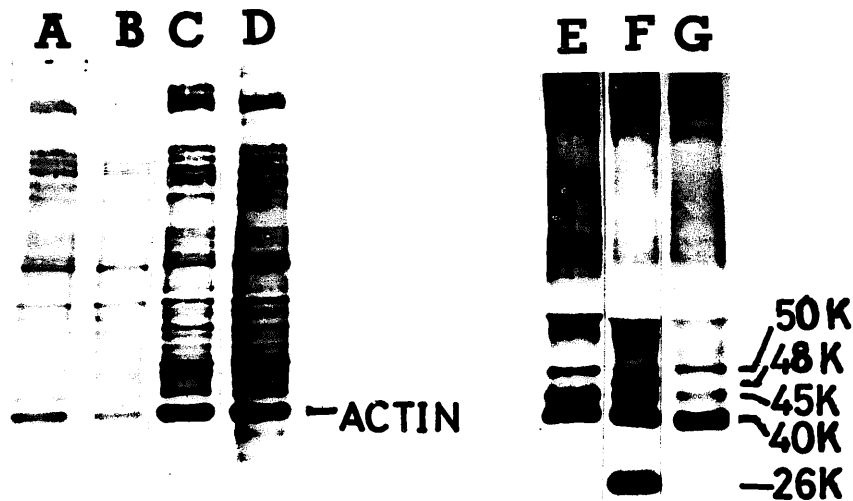
FIGURE 2. — Effect of DMSO on intracellular and secreted proteins. Quiescent and serum-stimulated MEF were obtained as described in *Materials and Methods*. Monolayers (25 cm²) of MEF were labelled with [³⁵S]methionine for 30 min. After a 30-min chase, the cells and media were collected and the proteins were electrophoresed in 5–18% polyacrylamide gradient gels containing SDS and the resolved proteins were fluorographed. Lanes A–D show the profiles of intracellular proteins: A, proteins of quiescent cells; B, proteins of quiescent cells treated with DMSO; C, proteins of serum-stimulated cells; D, proteins of FCS–DMSO treated cells. Lanes E–G depict the profiles of secreted proteins: E, secreted proteins of quiescent cells; F, secreted protein of serum-stimulated cells; G, secreted proteins of FCS–DMSO treated cells.
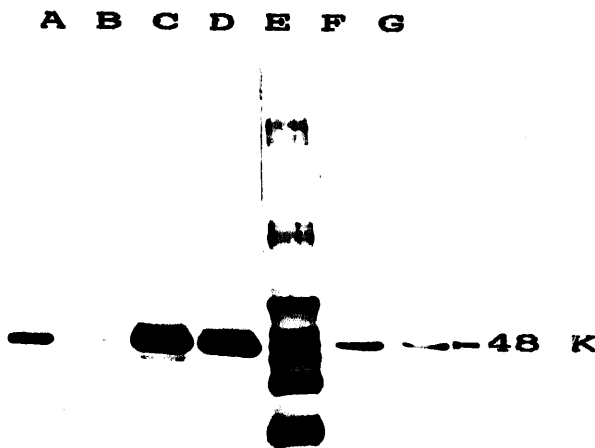

FIGURE 3. — Identification of 48 kDa protein as PAI-1. Quiescent cells were stimulated with 20% FCS and labelled with 50 μCi of [³⁵S]methionine for 30 min in serum-free medium. The matrix proteins were isolated and immunoprecipitations were performed as described [17, 18]. A, matrix proteins of quiescent cells. B, matrix proteins of quiescent cells treated with DMSO. C, matrix proteins of serum-stimulated cells. D, matrix proteins of serum-stimulated cells treated with DMSO. E, conditioned medium proteins of serum-stimulated cells F, conditioned medium proteins of serum-stimulated cells immunoprecipitated with PAI-1 antibody. G, matrix proteins from serum-stimulated cells immunoprecipitated with PAI-1 antibody.

The identity of the 26 kDa DMSO sensitive non-matrix protein was not established in this study.

Effect of DMSO on intracellular proteins

Under the conditions of inhibition of the extracellular levels of the 48 kDa and the 26 kDa proteins, DMSO did not preferentially inhibit the synthesis of any intracellular protein in quiescent or stimulated cells (Fig. 2 A–D), though the total protein synthesis was inhibited substantially (Fig. 8). It is difficult to monitor the level of the 48 kDa intracellular protein corresponding to the DMSO inhibited 48 kDa secreted protein from PAGE patterns since this was a minor band flanked closely by other protein bands. Therefore, indirect immunofluorescence based flow cytometric quantitation was carried out using antibodies to PAI-1. The results (Table I) indicate higher intracellular levels of this protein in FCS + DMSO treated cells than those observed in cells stimulated with FCS alone. These experiments in combination with the observations of reduced amounts of the 48 kDa protein in the matrix of DMSO treated cells imply that the polar solvent interferes with the transport of this protein into the medium.

Effect of DMSO on DNA synthesis

Addition of medium containing 20% FCS to quiescent mouse embryo fibroblasts, induced DNA synthesis and the synthesis reached a peak at 16–18 hr after the addi-
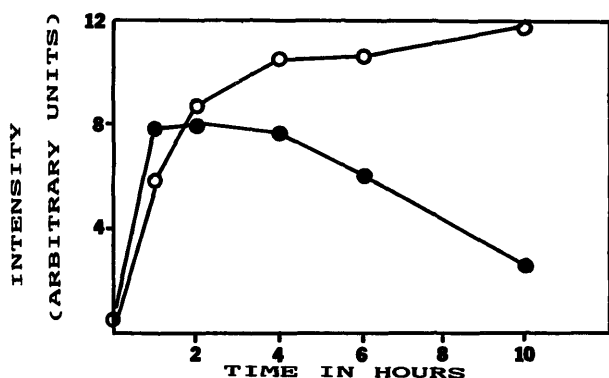
FIGURE 4. — Kinetics of inhibition of the matrix-associated 48 kDa protein levels by DMSO. Quiescent cells were labelled with [^{35}S]methionine at different periods after treating with FCS (o) or FCS + DMSO (●). The proteins were analysed by SDS PAGE and fluorographed. The levels of the matrix-associated 48 kDa protein were quantitated by computer assisted densitometric scanning and the relative areas under the 48 kDa protein were used for the graph.

tion of the medium (Fig. 5). At this point, 80% inhibition of DNA synthesis was observed in the FCS + DMSO treated cells. Further, there was a 6-hr delay before reaching the peak of DNA synthesis in FCS + DMSO treated cells. Addition of DMSO to epithelial cells (MK2) also resulted in the inhibition of DNA synthesis, though at reduced levels. Quiescent MK2 cells that were treated with medium containing 20% FCS + DMSO synthesised DNA only by 50% of that observed in cells treated with FCS alone.

In the studies described here, cells were treated with 2% DMSO; this concentration inhibited DNA synthesis by 90% (Fig. 6). Higher concentrations of DMSO (5–10%) resulted in complete abolition of serum-stimulated DNA synthesis. Cells treated with FCS + 5–10% DMSO did not show any increase in DNA synthesis over the background levels observed in quiescent cells. However, these high concentrations of the solvent were avoided due to possible deleterious effects on the cells. Serum-stimulated cells treated with 2% DMSO for 6 hr were found to be completely viable upon removal of DMSO and subsequent growth in serum-containing medium. Therefore, in all the studies described here, this concentration (2%) of DMSO was used.

Addition of DMSO during the early periods of serum-stimulated growth had marked effects on DNA synthesis in MEF (Fig. 7A) and MK2 (Fig. 7B) cells. Presence of DMSO up to 30–60 min of serum stimulation had drastic inhibitory effect on DNA synthesis in MEF and MK2 cells. DMSO added to cells at later stages of serum-stimulated growth showed little effect on DNA synthesis (Fig. 7A and 7B) although cell enlargement was evident (results not shown).

When [^{3}H]thymidine uptake was monitored, it was found that treatment of FCS stimulated cells with 2% DMSO resulted in 40–45% inhibition of thymidine incorporation into the cells. The DMSO-mediated inhibition of DNA synthesis described above cannot be correlated solely with the inhibition of thymidine uptake since (a) the DNA synthesis was inhibited to a higher extent that thymidine uptake and (b) the DNA synthesis was not

inhibited significantly when the cells were treated with DMSO at the later stages (3 hr) of mitogenic stimuli (Fig. 7 A).

Effect of DMSO on protein synthesis

Incorporation of [^{35}S]methionine into total proteins of serum-stimulated cells that were treated with DMSO is shown in Figure 8. Incorporation of labelled methionine into intracellular and secreted proteins was affected when DMSO was added to the cells at early stages of serum-stimulated growth. Treatment of cells with DMSO upto 30 min after serum stimulation had a more severe inhibitory effect on total protein synthesis than when DMSO was added at later stages of serum stimulation.

Effect of DMSO on RNA synthesis

Since the synthesis of early RNAs was among the crucial events of cell proliferation [19–21]. RNA synthesis was monitored in both DMSO treated and untreated quiescent cells stimulated to grow by the addition of 20% FCS. The results indicate that RNA synthesis during the early period of mitogenic response was inhibited by 85% (Fig. 9). Since the RNA synthesis was monitored in cells that were pulse-labelled for 30 min with [^{3}H]uridine, the inhibition may reflect a reduction in the synthesis of mRNAs. Although it is known that during the first few hours of serum stimulated growth, RNAs responsible for cell-cycle progression are synthesised [19], the specificity of inhibition by DMSO of particular species of mRNAs is to be determined.
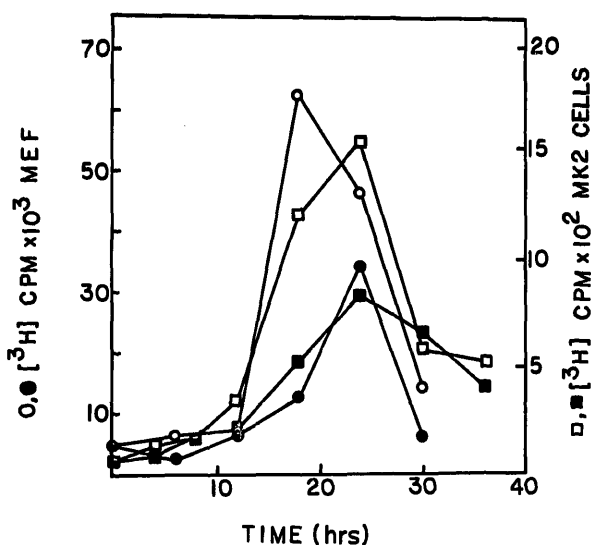

FIGURE 5. — Effect of DMSO on DNA synthesis. Quiescent cultures of MEF or MK2 cells were pulse-labelled for 30 min with [^{3}H]thymidine at different intervals after addition of 20% FCS or 20% FCS plus 2% DMSO. Cells were scraped and collected in PBS and the trichloroacetic acid precipitable counts were determined. o, FCS stimulated MEF; ●, FCS + DMSO treated MEF; □ FCS stimulated MK2 cells; ■, FCS + DMSO treated MK2 cells.
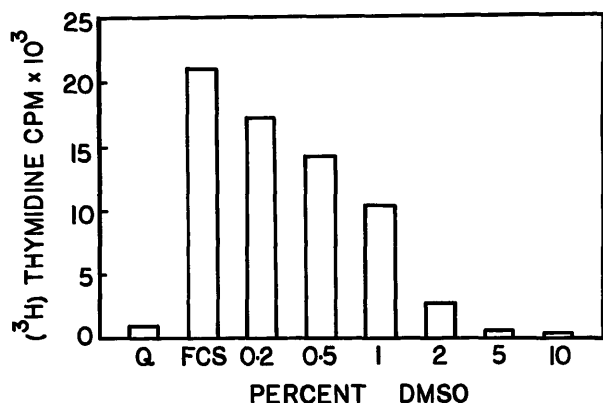
FIGURE 6. – Effect of DMSO concentration on DNA synthesis in serum-stimulated MEF. Quiescent cultures were stimulated with different concentrations of DMSO. The quiescent (Q), serum-stimulated (FCS) and DMSO treated cells were labelled for 30 min with 10 μCi of [³H]thymidine. Trichloroacetic acid precipitable counts were determined The numbers below the bars indicate the percent of DMSO used.

is increased by plasminogen activator (PA) which converts the proenzyme plasminogen to the active form plasmin [27]. Matrix deposition of PAI could have important biological consequences resulting in the inhibition of proteolysis. Presence of PAI on the cell membrane may inhibit the plasminogen activator activity thus preventing the pericellular proteolysis. Transfer of PAI from the membrane and deposition in the matrix may relieve the inhibition by PAI of the activity of the membrane associated PA; this might enable the activation of membrane bound plasminogen and trigger mitogenic stimuli by proteolytic cleavage (activation) of growth factors and (or) their receptors.
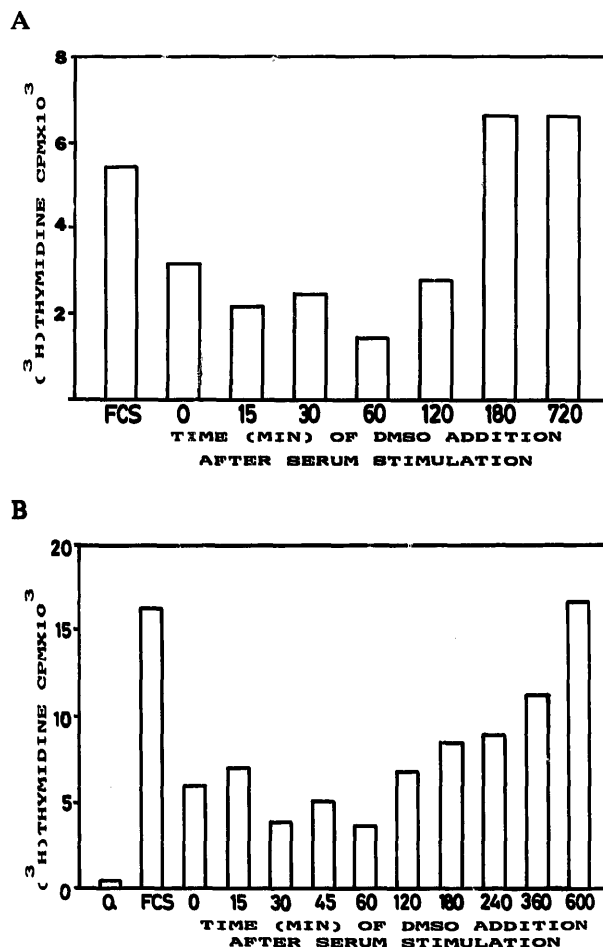
DISCUSSION

Our studies on the effect of DMSO on mouse embryo fibroblasts show that it affects the morphology, inhibits the synthesis of RNA, DNA and proteins. The extracellular levels of two proliferation-specific polypeptides of M_r 48000 and 26000 were also affected by DMSO. The most dramatic inhibitory effects of DMSO were observed at the early phases of mitogenic stimuli, within 30–45 min of serum-stimulated growth of mouse embryo fibroblasts.

The changes in the cell shape and DNA synthesis were shown to be related phenomena [22]. The enlargement of cells in the G1 phase of the cells was correlated with DNA synthesis in mammalian cells [23]. In transformed cells, DMSO treatment resulted in conferring non-malignant phenotype [2–4]. Our results on the morphological changes show increased spreading of cells upon DMSO treatment (Fig. 1). DMSO-mediated cell enlargement was evident at all stages of serum-stimulated growth, while the polar solvent inhibited DNA synthesis only when it was added at the early stages of serum-stimulated growth. These results imply that the DMSO-mediated cell enlargement and the inhibition of DNA synthesis were unrelated events in mouse embryo fibroblasts.

Our previous studies on secretory proteins showed that the 48 kDa and the 26 kDa proteins were proliferation-specific. These proteins were induced by mitogens and cycloheximide [24, 25]. The results presented here show that the 48 kDa protein is a serum-induced protein and is related to endothelial type plasminogen activator inhibitor (Fig. 2). Addition of DMSO to quiescent and serum-stimulated cells reduced the level of the PAI-1 related 48 kDa protein in the medium and interfered with its deposition in the extracellular matrix.

Cells in culture synthesise proteinases and their activators and inhibitors [26]. Plasminogen is one of the proteases secreted by the cells. The activity of this enzyme

A

B

FIGURE 7. – Effect of DMSO on DNA synthesis in MEF (A) and MK2 (B) cells pretreated with 20% FCS. Quiescent cultures were stimulated to grow by the addition of 20% FCS. DMSO was added to cultures at different periods after serum stimulation. The numbers below the bars indicate the time (in min) after serum stimulation and DMSO was added at these points. The DMSO treated and control dishes were labelled for 30 min with 10 μCi of [³H]thymidine at 18 hr after serum stimulation. Cells were scraped and suspended in PBS and precipitated with equal volume of 10% trichloroacetic acid and the acid precipitable counts were determined. The letters Q and FCS below the bars indicate the extent of DNA synthesis in quiescent (Q) and serum-stimulated (FCS) cells that were not treated with DMSO.
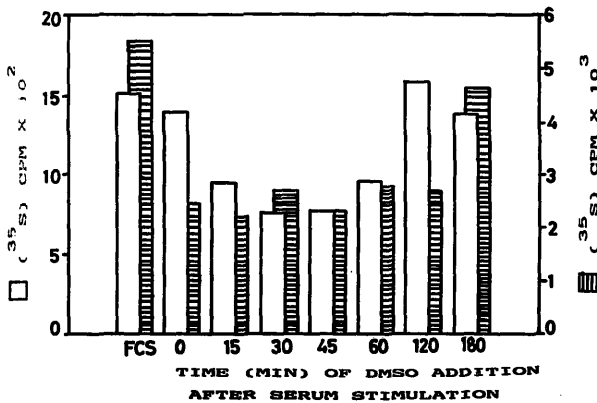
FIGURE 8. – Effect of DMSO on protein synthesis in cells pretreated with 20% FCS. Quiescent cultures of MEF were stimulated to grow by the addition of 20% FCS. DMSO was added to cultures at different periods after serum stimulation. FCS, serum-stimulated cells. The numbers below the bars indicate the time (in min) of DMSO addition after serum stimulation. The DMSO-treated and control monolayers (25 cm²) were labelled with 50 µCi of [³⁵S]methionine in 5 ml of Hank's balanced salt solution for 30 min. After a 30-min chase incubation in the serum-free medium, the chase medium was collected and the cells were lysed with electrophoresis sample buffer. Aliquots of medium and cell lysates were precipitated with trichloroacetic acid and acid precipitable counts were determined. ☐, Conditioned medium proteins. ☐, Intracellular proteins.
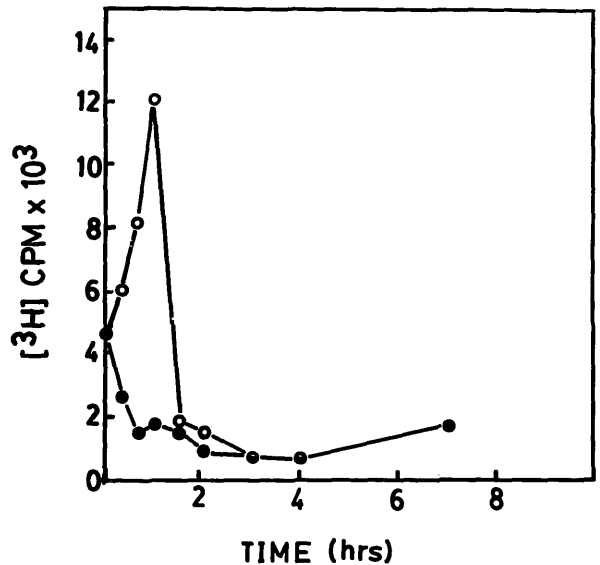

FIGURE 9. – Effect of DMSO on RNA synthesis. Quiescent cultures of mouse embryo fibroblasts were labelled with 10 µCi of [³H]uridine for 30 min at different periods after addition of 20% FCS or 20% FCS+DMSO. o, FCS stimulated cells; •, FCS+DMSO treated cells.

Accumulation of PAI-1 on the cell growth substratum has been reported by Pollanen et al. [28]. PAI may be involved in the production of structural changes in cells apart from inhibiting the plasminogen activator. Quigley [27] showed changes in morphology in chick embryo fibroblasts transformed with Rous sarcoma virus as a result of enhanced PA activity. Addition of synthetic PAI inhibited these morphological changes. Laiho et al. [17] have reported the enhanced deposition of PAI-1 on the ECM of human lung fibroblasts exposed to TGF β. These studies indicate that ECM proteins play a major role in a number of phenomema such as tissue integrity, cell adhesion, metastasis and cell migration [29].

The primary effect of DMSO on cultured cells appears to be on the cell membrane and such an effect was shown to interfere with the binding of EGF with its receptor [12]. Alteration of membrane conformation may change the transport properties of the membrane. The higher intracellular levels of PAI-1 in FCS+DMSO treated cells and lower levels in the medium of the same cells may reflect an impairment of transport of this protein from the cell to the medium as the result of DMSO mediated change of membrane structure.

Synthesis of new mRNAs is required for the progression of quiescent cells to enter through G1 [19]. Pardee et al. [30] and Schneiderman et al. [31] reported that protein synthesis in the early G1 phase of the cell cycle is directed by newly made mRNAs which are required for cell growth. Our results show that the early RNA synthesis is inhibited by DMSO treatment. The proto-oncogenes c-

myc and c-fos are induced in quiescent cells at the early periods of addition of serum or growth factors [32, 33]. Apart from proto-oncogenes, several other cellular proteins were found· to be regulating the early G1 phase [34–40]. Here we have shown the inhibition of the extracellular levels of two early growth response proteins (the 48 kDa and 26 kDa proteins) by DMSO. It is to be determined whether DMSO affects the synthesis of mRNAs of these proteins and that of other early growth response genes including c-fos and c-myc.

Addition of serum or growth factors to quiescent cells induces several changes. An increase in the phosphotidylinositol turnover, phosphorylation of proteins, autophosphorylation of growth factor receptors, increase in intracellular pH and Ca^{2+}, rearrangement of actin bundles, increase in nutrient transport and enhanced RNA synthesis are some of the consequences of serum-stimulated growth [34,41]. DMSO has been reported to stimulate tyrosine residue phosphorylation of epidermal growth factor (EGF) receptor and subsequently shown to affect the binding of EGF to its receptor [12]. The DMSO-mediated reduced binding of EGF to its receptor may result in the inhibition of DNA synthesis. It is to be determined whether the DMSO sensitive 48 kDa and 26 kDa proteins influence any of the above growth-related events. Since DMSO treatment of cells at late stages of mitogenic stimuli did not inhibit DNA synthesis and other early events, we presumed that the polar solvent may not have any effect when added after the early mitogenic signals were conveyed, and this was indeed what we have observ-

ed. We conclude that once the DMSO-sensitive early mitogenic signals have been conveyed, DMSO treatment at later stages of mitogenic stimuli will be ineffective in inhibiting the signal(s). Dimethyl sulfoxide is often used in ointments and rubs as solvent and sometimes as home remedy for arthritis. Since treatment of cells with DMSO interferes with vital processes of growth as shown here, caution must be exerted on its use in medical formulations.

ACKNOWLEDGEMENTS

REFERENCES

1 Friend C., Scher W., Holland J.G. & Sato T. (1971) Haemoglobulin synthesis in murine virus-induced leukemia cells *in vitro*: stimulation of erythroid differentiation by dimethyl sulfoxide. *Proc. Natl Acad. Sci. USA* 68, 378–382

2 Borenfreund E., Steinglass M., Korngold G. & Bendich A. (1975) Effect of dimethyl sulfoxide and dimethylformamide on the growth and morphology of tumor cells. *Ann. NY Acad. Sci.* 243, 164–171

3 Higgins P.J. (1982) Alterations in cellular morphology, proliferative rate, and peptide composition accompany dimethyl sulfoxide enhanced liver protein synthesis by hepatoma cells. *Cell Mol. Biol.* 28, 299–302

4 Lampugnani M.G., Pedenovi M., Niewiarowski A., Casali M., Donati M.B., Corbascio G.C. & Marchislo P.C. (1987) Effects of dimethyl sulfoxide (DMSO) on microfilament organisation, cellular adhesion, and growth of cultured mouse B16 melanoma cells. *Exp. Cell Res.* 172, 385–396

5 Ossowski L. & Belin D. (1985) Effect of dimethyl sulfoxide on human carcinoma cells, inhibition of plasminogen activator synthesis, change in cell morphology, and alteration of response to cholera toxin. *Mol. Cell Biol.* 5, 3552–3559

6 Takenaga K. (1984) Enhancement of lung colonising ability of cloned low-metastatic Lewis lung carcinoma cells by treatment with highly polar solvents. *Int. J. Cancer* 34, 83–89

7 Dexter D.L. (1977) N-N-dimethylformamide induced morphological differentiation and reduction of tumorigenecity in cultured mouse rhabdomyosarcoma cells. *Cancer Res.* 37, 3136–3140

8 Dexter D.L., Barbosa J.A. & Calabresi P. (1979) N-N-dimethylformamide induced alteration of cell culture characteristic and loss of tumorigenicity in cultured human colon carcinoma. *Cancer Res.* 39, 1020–1025

9 Higgins P.J. & Borenfreund E. (1980) Enhanced albumin production by malignantly transformed hepatocytes during *in vitro* exposure to dimethyl sulfoxide. *Biochem. Biophys. Acta* 610, 174–180

10 Huberman E., Heckman D. & Lagenbach R. (1979) Stimulation of differentiation functions in human melanoma cells by tumor promoting agents and dimethyl sulfoxide. *Cancer Res.* 39, 2618–2624

11 Rudland P.S., Davis A.T. & Warburton M.J. (1982) Prostoglandin induced differentiation or DMSO induced differentiation: reduction in neoplastic potential of a rat mammary tumor stem cell line. *J. Natl Cancer Inst.* 69, 1083–1093

12 Earp H.S., Lin Q. & Blaisdell J. (1984) Dimethyl sulfoxide decreases specific EGF binding. *J. Cell Biochem.* 26, 221–230

13 Barra R., Hicks H.R., Koch M.R. & Lea M.A. (1978) Stimulatory effect of dimethyl sulfoxide on [^{3}H]thymidine incorporation into DNA in Novikoff hepatoma cells. *Int. J. Biochem.* 9, 389–394

14 Collins S.J., Ruscetti F.W., Gallagher R.E. & Gallo R.C. (1978) Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl sulfoxide and other polar solvents. *Proc. Natl Acad. Sci. USA* 75, 2458–2462

15 Rubin R.A. & Earp H.S. (1983) Solubilisation of EGF receptor with Triton X-100 alters stimulation of tyrosine residue phosphorylation by EGF and dimethyl sulfoxide. *Science* 219, 60–62

16 Nagashunmugam T. & Shanmugam G. (1987) S-phase embryo fibroblasts secrete varying amounts of a 45000 dalton protein. *Cell Biol. Int. Rep.* 12, 147–155

17 Laiho M., Saksela O., Andreasen P.A. & Keski-Oja J. (1986) Enhanced production and extracellular deposition of the endothelial-type plasminogen activator inhibitor in cultured human lung fibroblasts by transforming growth factor β. *J. Cell Biol.* 103, 2403–2410

18 Chackalaparampil I., Banerjee D., Poirier Y. & Mukherjee B.B. (1985) Altered processing of a major secreted phosphoprotein correlates with tumorigenecity in Rous sarcoma virus transformed mammalian cells. *J. Virol.* 53, 841–850

19 Baserga R., Waechter D.E., Soprano K.J. & Galanti N. (1982) Molecular biology of cell division. *Ann. NY Acad. Sci.* 397, 110–120

20 Carpenter G. & Cohen S. (1979) Epidermal growth factor. *Annu. Rev. Biochem.* 48, 193–216

21 Getz M.J., Elder P.K., Benz E.W., Stephens R.E. & Moses H.L. (1976) Effect of cell proliferation on levels and diversity of poly-A containing mRNA. *Cell* 7, 255–265

22 Folkman J. & Moscona A. (1978) Role of cell shape in growth control. *Nature* 273, 345–349

23 Killander D. & Zetterberg A. (1965) A quantitative cytochemical investigation of the relationship between cell mass and initiation of DNA synthesis in mouse embryo fibroblasts *in vitro*. *Exp. Cell Res.* 40, 12–20

24 Subramaniam M. & Shanmugam G. (1985) Intracellular and secreted proteins of density-inhibited, serum-arrested and proliferating mouse embryo cells. *Cell Biol. Int. Rep.* 9, 51–60

25 Subramaniam M. & Shanmugam G. (1987) Secreted proteins of quiescent, serum-stimulated and over-confluent mouse embryo fibroblasts. *J. Bioscience* 12, 281–87

26 Saksela O. (1985) Plasminogen activation and regulation of pericellular proteolysis. *Biochem. Biophys. Acta* 823, 35–65

27 Quigley J.P. (1986) Association of a protease (plasminogen activator) with a specific membrane fraction isolated from transformed cells. *J. Cell Biol.* 71, 472–486

28 Pollanen J., Saksela O., Salonen E.M., Andreasen P.A., Nielsen L., Dano K. & Vaheri A. (1987) Distinct localisation of urokinase-type plasminogen activator and its type 1 inhibitor under cultured human fibroblasts and sarcoma cells. *J. Cell Biol.* 104, 1085–1096

29 Buck C.A. & Horwitz A.F. (1987) Cell surface receptors for extracellular matrix molecules. *Annu. Rev. Cell Biol.* 3, 179–205

30 Pardee A.B., Dubrow R., Hamlin J.L. & Kletzien R.F. (1978) Animal cell cycle. *Annu. Rev. Biochem.* 47, 715–750

31 Schneiderman M.H., Dowey W.C. & Highfield D.P. (1971) Inhibition of DNA synthesis in synchronised Chinese Hamster cells treated in G1 with cycloheximide. *Exp. Cell Res.* 67, 147–155

32 Greenberg M.E. & Ziff E.B. (1984) Stimulation of 3T3 cells induced transcription of c-fos proto-oncogene. *Nature* 311, 433–438

33 Kelly K., Cochran B.H., Stiles C.D. & Leder P. (1983) Cell specific regulation of the c-myc gene by lymphocyte mitogens and platelet-derived growth factor. *Cell* 35, 603–610

34 Cochran B.H. (1985) The molecular action of platelet derived growth factor. *Adv. Cancer Res.* 45, 183–216

35 Foster D.N., Schmidt L.J., Hodgson C.P., Moses H.L. & Getz M.J. (1982) Polyadenylated RNA complementary to a mouse retrovirus like multigene family is rapidly and specifically induced by EGF stimulation of quiescent cells. *Proc. Natl Acad. Sci. USA.* 79, 7317–7321

36 Hirschhorn R.R., Aller P., Yuan Z.A., Gibson C.W. & Baserga R. (1984) Cell-cycle specific cDNAs from mammalian cells temperature sensitive for growth. *Proc. Natl. Acad. Sci. USA* 81, 6004–6008

37 Lau L.F. & Nathans D. (1985) Identification of set of genes expressed during the Go/G1 transition of cultured mouse cells. *EMBO. J.* 4, 3145–3151

38 Linzer D.I.H. & Nathans D. (1983) Growth-related changes in specific mRNAs of cultured mouse cells. *Proc. Natl Acad. Sci. USA* 80, 4271–4275

39 Subramaniam M. & Shanmugam G. (1986) A serum-induced 29 Kd protein of mouse embryo fibroblasts is tightly bound to the chromatin. *Cell Biol. Int. Rep.* 10, 323–329

40 Subramaniam M. & Shanmugam G. (1986) Effect of cycloheximide and growth factors on gene expression in quiescent mouse embryo fibroblasts. *J. Cell. Physiol.* 126, 47–52

41 Rozengurt E. (1986) Early signals in the mitogenic response *Science* 234, 161–166