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COMMENTARY

EFFECTS AND POSSIBLE MECHANISM OF ACTION OF DIMETHYLSULFOXIDE ON FRIEND CELL DIFFERENTIATION*

CHARLOTTE FRIEND and HERBERT A. FREEDMAN†
The Center for Experimental Cell Biology, The Mollie B. Roth Laboratory,
Mount Sinai School of Medicine of the
City University of New York, New York, NY, U.S.A.

Dimethylsulfoxide (DMSO) is a dipolar, aprotic organic compound [1], having a high dielectric constant [2]. It is, therefore, an excellent solvent for most organic compounds [3] and is active in biological systems. The finding that DMSO is capable of stimulating erythroid differentiation of malignant Friend erythroleukemia (FL) cells *in vitro* [4, 5] brought into prominence still another activity of this versatile compound. New vistas to studies relating the mechanisms controlling differentiation and malignancy were thus opened.

Under normal conditions, cell division is regulated by a complex set of mechanisms controlling each step in the course to maturation. Undifferentiated or "immature" cells generally divide, giving rise to progeny which differentiate along specific pathways. As the maturation of these progeny cells proceeds, there is believed to be a "feedback" to the original undifferentiated parental cell (stem cell), halting further division. Thus, when cells reach the terminal stage of differentiation, they lose the capacity to multiply further. In contrast, one of the fundamental properties of malignancy is the maintenance of undifferentiated neoplastic cells which continue to divide. Failure of these cells to differentiate results in an ever-increasing population of immature cells no longer responsive to the growth control signals programming the maturation of normal cells. Accordingly, a block in differentiation may lead to the rapid and self-perpetuating activity characteristic of malignant disease.

Since the regulatory mechanisms controlling differentiation and neoplastic growth are believed similar [6–11], a system in which the differentiation of malignant cells can be induced would allow study of the mechanisms not only controlling malignancy, but regulating normal growth and differentiation as well. Such a model system is provided by FL cells, which consist of a population of malignant, undifferentiated primitive red cell precursors. Under certain conditions, these cells can be stimulated to differentiate along the erythrocytic pathway with concomitant decrease in malignancy [4, 5].

Permanent lines of FL cells which continue to synthesize virus [12, 13] have been established *in vitro* [14–17]. Such cell lines represent a population of chronically infected erythroid precursor cells arrested at the proerythroblast level of development. The cells grow in suspension with a doubling time of 12–20 hr depending on the line, and in general have a small percentage of spontaneously differentiating cells. A few variant clones, however, have been found to have a high baseline of spontaneously differentiating cells [18]. Differentiating FL cells mature to the level of orthochromatic normoblast and synthesize hemoglobin [19–22].

When FL cells are grown in the presence of 1–2% (0.14 to 0.28 M) DMSO for 4–5 days, approximately 90 percent of the cells are stimulated to differentiate to the normoblast level [4]. The series of changes occurring during this period parallel those occurring during the course of normal red cell maturation [21]: hemoglobin accumulates [19–24], chromatin condenses and cell division ceases [14–16, 25–27], and the activity of enzymes involved with the *de novo* biosynthetic pathway is decreased [28]. Moreover, there is: (1) increased delta-aminolevulinic acid synthetase activity [29, 30] and heme synthesis [4, 20, 22], (2) accumulation of globin mRNAs [31–34] and large amounts of adult mouse strain-specific globins [16, 23], (3) expression of erythrocytic membrane-specific antigens [35], and (4) increased carbonic anhydrase activity [36, 37]. With regard to normal cell differentiation, DMSO has been shown to stimulate heme synthesis in the dissociated–reaggregated yolk sac of normal avian embryo (quail) cells during the course of blood island formation [38], to enhance the sensitivity of certain normal erythroid precursor cells to low doses of erythropoietin, and to induce differentiation in human lung cells [39] and murine neuroblastoma cells [40, 41].

Although other polar organic solvents [42–46], short chain fatty acids [47, 48], hemin [49], several purines and purine analogues [50], and a number of metabolic inhibitors [51] have also been found to be potent inducers of erythroid differentiation in FL cells, DMSO has been the inducer most frequently studied. While the basic mechanism by which these compounds stimulate differentiation is not clear, the action of DMSO has been attributed to a number of its many properties. It penetrates efficiently through biological membranes [52, 53], and so is an exceptionally good carrier of drugs [54–56], electrolytes and non-electrolytes [57], polar and polarizable molecules [58], dyes [59], bacteriocides [60] and

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† Department of Pathology, State University of New York, Downstate Medical Center, Brooklyn, NY. Special Fellow of the Leukemia Society of America, Inc.

nutrients [61]. It has also been reported to inhibit the activity of several enzymes such as cholinesterase [62], urease and chymotrypsin [63], phosphomonoesterase, β-galactosidase, peroxidase, catalase and dehydrogenase [64], to increase the activity of deoxyribonuclease [65], and to activate lysosomes [66], resulting in the release of acid phosphatase [67]. It is believed that DMSO effects changes in enzyme activity by producing conformational changes in the protein molecules themselves, resulting in altered interactions between the active site of the enzyme and substrate molecules [68]. Similar changes in structural proteins have been suggested to increase the permeability of cell membranes [69] and to alter the secondary structure of DNA and RNA [70] by denaturation [71].

At low concentrations of DMSO, the uptake of both zinc and O_2 is altered [72], but enzyme activity is reportedly unchanged [73, 74]. This effect on O_2 uptake appears earlier and is greater than the effect on proteins and nucleic acids. Thus, it has been suggested that DMSO alters cellular metabolism by interfering with the respiratory process. This would result in a lack of energy of the phosphate bond of ATP which, in turn, could affect both transcription and protein synthesis [75]. Archer *et al.* [76] have reported that the metabolic alterations induced by DMSO are probably caused by interference with cell membrane bound electron transfer systems.

The results of a number of studies point to the involvement of membrane function in the action of DMSO. Local anesthetics at concentrations that do not affect cellular proliferation [77] have been reported to inhibit the effect of inducers of FL cell differentiation. These anesthetic amines, which have been shown to increase the fluidity of artificial lipid bilayers [78, 79] and biological membranes [80], increase the permeability of cell membranes. Similarly, the fungicide Amphotericin B increases membrane permeability and inhibits DMSO-induced FL cell differentiation [81]. DMSO, as well as other dipolar solvents capable of acting as inducers of erythroid differentiation, have also been shown to produce a decrease in the fluidity of phospholipid membranes which can be reversed by local anesthetic amines [82]. Further, it has been suggested that the effect of DMSO and other di-polar aprotic solvents on membrane permeability results from the disruption of water structures [83], with consequent dehydration of the biomembrane and alteration of protein structures [84]. Bernstein *et al.* [77] have proposed that: "Changes in membrane structure, and, in particular, in membrane fluidity, can alter the pattern of gene expression in FL cells, and possibly in other differentiating systems."

N,N'-dimethylformamide (DMF), like DMSO, is a dipolar aprotic solvent which affects membrane-bound enzymes and induces erythroid differentiation in Friend cells [42]. Both of these compounds inhibit N^+,K^+-dependent ATPase and stimulate Ca^{2+}-transport ATPase [85]. These activities would account for the reported loss of K^+ and influx of Na^+ in human red cells treated with DMSO [83]. Bernstein *et al.* [86] have shown that the cardiac glycoside, ouabain, which binds specifically to and inhibits the plasma membrane enzyme Na^+,K^+-ATPase, induces erythroid differentiation in FL cells. In fact, these workers observed heme-containing cells 12–24 hr earlier when ouabain rather than DMSO was used as inducer. Since (1) ouabain induced hemoglobin synthesis at concentrations which inhibited K^+ uptake by approximately 30–50 percent, and (2) the background level of hemoglobin-containing cells was consistently 2- to 3-fold higher in low K^+ than in normal K^+ medium, it was suggested that changes in the intracellular concentration of K^+ ions may be involved in the induction of FL cell differentiation. This is consistent with earlier reports implicating the intracellular concentration of K^+ in the control of cell growth [87], early embryonic development [88], oöcyte maturation [89, 90] and the mitogenic effect of phytohemagglutinin on lymphocytes [91, 92]—an effect which is inhibited by DMSO [93].

In addition to effects on membrane functions, DMSO and other cryoprotective agents have been shown to cause the release of large amounts of both nucleic acids (primarily 'soluble' RNA), xanthine, hypoxanthine and proteins from Ehrlich tumor cells [94, 95]. The origin of the two purines was not clear. They could have arisen from the catabolism of nucleic acids, free purines, purine nucleosides and/or purine nucleotides. Be that as it may, at least one of these products—the purine, hypoxanthine—and its direct analogues, 6-thioguanine and 6-mercaptopurine, have been found to be inducers of erythroid differentiation in the FL cell system [50]. Induction does not require purine incorporation into DNA or RNA, because all three compounds can induce erythroid differentiation in hypoxanthine-guanine-phosphoribosyltransferase-negative (HGPRT-) FL cell lines.

DMSO also inhibits DNA synthesis in cultured S-180 tumor cells [96] and, at concentrations of 1%, decreases the mitotic index of normal dividing cells [75]. It has been suggested that the induction of differentiation in FL cells by DMSO requires some cell cycle-dependent process [97, 98]. In the FL cell system, DMSO causes an initial lag in growth, but does not affect cell viability [4], mitotic rate or DNA content [99]. Irreversible commitment of FL cells to erythroid differentiation has been reported to occur after as little as 12–18 hr of continuous exposure to DMSO [27]. However, globin mRNA is only detectable after 24 hr [31] and heme and globin synthesis after a period of 2–3 days [4, 42]. This temporal sequence may be related to the observation that 14–22 hr is needed for equilibration of the intracellular space with DMSO [97]. These observations are consistent with previous studies indicating that, in the FL cell system, at least two rounds of DNA synthesis in the presence of DMSO are required for the induction of hemoglobin [97, 98]. There appears to be no prerequisite for DNA synthesis when butyric acid is used as the inducer [48].

In addition, DMSO has been reported to affect chromatin structure. Chromatin is organized in discontinuous subunits [100–103], forming a hydrophobic core of apolar histones [103–105]. Thus, changes in the physicochemical properties of the environment could influence the hydrophobic interactions in the chromatin subunits, altering the restriction of histones on DNA transcription [106]. Recently, Strätling [106] has shown that DMSO and

other polar organic compounds, capable of inducing erythroid differentiation, increased RNA synthesis on isolated chromatin from FL cells. Separation of the isolated chromatin into template active and inactive fractions showed that DMSO increased RNA synthesis to a greater extent on the transcriptionally restricted (template inactive) than on the template active portions. This effect was suggested to result from a weakening of the apolar interactions between histones in chromatin subunits partially releasing transcription from the constraints of histones. Earlier, Travers [107] had shown that both DMSO and glycerol stimulated the rate of RNA synthesis in a cell-free system by a direct effect on the DNA template. Recently, a new, non-histone chromosomal protein, whose role is yet to be defined, has been detected in DMSO-stimulated FL cells [108].

When FL cells are treated with the halogenated pyrimidine bromodeoxyuridine [BUdR], induction of erythroid differentiation by both DMSO and DMF is inhibited [42, 109, 110]. This inhibition can be prevented by the addition of thymidine [42], which interferes with the incorporation of BUdR into DNA. BUdR substitutes for thymidine in DNA, substantially stabilizing it against thermal denaturation [111], and promotes tighter binding of chromosomal proteins [112, 113]. Since interphase chromatin consists of diffuse transcriptionally active and condensed transcriptionally inactive regions [114, 115], it has been suggested that the incorporation of BUdR into DNA results in a tightened DNA or DNA:protein structure, unable to promote new transcriptional activity [116–119]. On the contrary, DMSO, at a concentration of 1%, decreases the overall thermostability of chromatin, antagonizing the effects of BUdR substitution by allowing chromatin to transform to a more relaxed (extended) state [120]. Thus, DMSO promotes the expression of promoter–defective *gal* and λ operons, and increases the expression of the *gal* operon, even in the presence of active *gal* repressor [121].

In view of these findings, it has been suggested that, in the FL cell system, DMSO and related polar compounds may act by altering the conformation of DNA or the DNA:protein complex, producing a relaxation of chromatin structure [28, 43, 106], perhaps by disruption of hydrogen bonds [44]. Since gene expression in eukaryotes is closely related to chromatin conformation [113], a change in chromatin structure may alter transcription, causing a derepression of the erythroid differentiation program. Aviv *et al.* [122] have shown that the accumulation of globin mRNA in DMSO-stimulated Friend cells does indeed result from increased transcription (i.e. derepression) of the globin gene. The latest studies indicate that DMSO and other inducers may have a profound, though indirect, effect on DNA. The single-strand scissions found in DMSO-treated cell DNA may mark an early step in the control of differentiation [123].* The detection of alterations in the structure of folded genomes in treated FL cells provides additional evidence that DMSO damages DNA.

While the effects of DMSO on differentiation in the FL cell system have been stressed, it should be mentioned that this compound also influences virus production [13, 124, 125]. Evidence points to the fact that the mechanisms controlling virus synthesis and differentiation are, however, under separate control. BUdR inhibits DMSO stimulation of erythroid differentiation in FL cells [42], but causes an enhancement of virus production in cells treated with a combination of both compounds [125, 126]. Moreover, variant clones, which are DMSO resistant in that they do not undergo erythroid differentiation, can be stimulated by DMSO to synthesize increased quantities of virus [125, 127]. Clones which are highly responsive to DMSO-induced differentiation may not show DMSO-enhanced virus production.

We have attempted to put into perspective the broad spectrum of effects induced by DMSO in biological systems. This compound has been shown to primarily: (1) affect enzyme systems, (2) alter the secondary structure of DNA and RNA, (3) alter O_2 uptake, (4) decrease membrane permeability and fluidity, (5) inhibit Na^+,K^+-dependent ATPase and stimulate Ca^{2+}-transport ATPase, (6) inhibit DNA synthesis, (7) cause scission in DNA and alterations in folded genomes, (8) increase RNA synthesis on transcriptionally restricted (template-inactive) fractions of isolated chromatin, and (9) decrease the overall thermostability of chromatin.

While any of the above effects of DMSO may be sufficient to stimulate erythroid differentiation of FL cells and/or virus synthesis, the basic mechanism(s) controlling these functions remain unclear. Indeed, in biological systems, these activities may be interrelated and/or occur in concert.

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