Volume 304, number 2,3, 198–200 FEBS 11151 June 1992

Inhibitors of polyamine biosynthesis affect the expression of genes encoding cytoskeletal proteins

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Received 13 April 1992

The polyamines are ubiquitous components of mammalian cells. Those compounds have been postulated to play an important role in different cellular functions including the reorganization of cytoskeleton associated with the cell cycle. In the studies reported here, it was found that inhibitors of polyamine biosynthesis, methylglyoxal-bis[quanylhydrazone] (MGBG) and difluoromethylornithine (DFMO), prevent mitogen-induced accumulation of mRNAs encoding major cytoskeletal components, β-actin and α-tubulin, in mouse splenocytes. These findings suggest mechanisms through which polyamines may exert their effects on the cytoskeleton integrity.

Polyamine; DFMO; MGBG; Mouse splenocyte; β-actin; α-tubulin mRNA; Cytoskeleton

1. INTRODUCTION

The polyamines, spermidine and spermine, and their precursor putrescine, are ubiquitous components of mammalian cells [1]. Although the physiological functions of these compounds are still not well understood, several studies have shown that normal cellular growth and division require polyamines [2–5]. Direct support for this notion has been provided by experiments in which polyamine synthesis was prevented by the application of inhibitors [6–8]. Methylglyoxal-bis[quanylhydrazone] (MGBG), an inhibitor of *S*-adenosylmethionine decarboxylase, and difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase, are most widely used drugs inhibiting key polyamine-biosynthesizing enzymes. DFMO and MGBG inhibited growth of many types of mammalian cells in culture (for review see [9]). More precise investigation of the effects of polyamine depletion on the cell cycle revealed the involvement of polyamines in cytoskeleton structure and cell division [10]. Moreover, Pohjanpelto et al. [11] observed the disappearance of 90% of actin filament bundles and lack of assembly of microtubules in DFMO-treated CHO cells. Similarly, Sunkara et al. [12] have reported cessation of cell division and an increase in the number of multinuclear cells in different mammalian cells treated with MGBG and α-methylornithine.

Oriol-Audit et al. [13,14] have shown in vitro that polyamines can promote polymerization of actin monomers into filaments and association of filaments into bundles. This polyamines-induced arrangement of actin filaments to form the contractible ring has been proposed as a mechanism of cytokinesis [15]. Other investigations showed that micro-injected polyamines cause a shortening of the division cycle in *Xenopus* eggs and induce cytokinesis of *Amoeba* [15].

Our previous studies have shown that exogenously added polyamines, putrescine and spermine, are capable to initiate the DNA synthesis of mouse T splenocytes and promote lymphoblast transformation and cell division. Additionally, both spermine and putrescine induced increases in expression of genes coding for major cytoskeletal proteins [16]. In this report we present a further study on the effects of polyamine inhibitors on the expression of genes encoding cytoskeletal proteins which has been carried out in order to understand the molecular basis of the relationship between polyamines and cytoskeleton.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

MGBG was from Sigma Chemical Company. DFMO was a generous gift from the Merrel Dow Research Institute. Stock solutions were prepared in RPMI 1640 medium, sterilized by membrane filtration and stored at −20°C. [*methyl*-^{3}H]Thymidine (specific activity 27 Ci/mmol) was from Amersham. All other chemicals used were of analytical grade and were obtained from standard commercial sources.

2.2. *Cell culture and drug treatment*

Splenocytes were obtained from mechanically disrupted spleens of 3-months-old Swiss albino mice. Lymphocytes were separated through Ficoll gradient centrifugation and next cultivated at a concentration of 2×10^2 cells/ml in RPMI 1640 medium supplemented with 5% of fetal bovine serum, 2 mM L-glutamine, 20 mM HEPES, 5×10^{-5} M β-mercaptoethanol and antibiotics. After 24 h of preincubation, T

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lymphocytes were treated with 5 μg/ml of concanavalin A and/or inhibitors of polyamine biosynthesis.

The cultures were monitored for the level of stimulation by measuring [*methyl*-^{3}H]thymidine incorporation into DNA (4 μCi/ml for 6-h pulse labeling) 66–72 h following Con A addition.

2.3. *Isolation and Northern analysis of RNA*

Total cellular RNA was extracted according to the method of Chomczynski and Sacchi [17]. RNA was quantified by measuring the absorbance at 260 nm. Quantification and integrity of each RNA sample were confirmed by analysis on agarose gels stained with ethidium bromide. Samples of RNA (15 μg) were fractionated in 1% agarose gel containing 2.2 M formaldehyde and blotted to nitrocellulose filters. For hybridization, filters were used only when mRNA levels were the same for all lanes. The filters were next hybridized with whole plasmids carrying gene probes ^{32}P-labelled by the random priming method with the Klenow fragment of DNA polymerase I (spec. act. 5–10 × 10^8 cpm/μg DNA). Prehybridization, hybridization and washing were as described by Messina et al. [18]. The filters were exposed to Kodak X-ray films with the use of intensifying screens at −70°C.

The β-actin and α-tubulin mRNA levels were measured densitometrically with the aid of a Shimadzu laser densitometer. Then the mRNA levels were compared after normalization according to densitometrically scanned pictures of 18 rRNA visible on nitrocellulose after blotting due to the staining with ethidium bromide. The mRNA levels have been expressed relatively to the level detected in nontreated cells.

The clones used in this work were: pHFa-1 human β-actin isolated by Gunning et al. [19]; mouse α-tubulin (obtained from Dr. E. Fuchs).

3. RESULTS AND DISCUSSION

The specific inhibitors of polyamine biosynthesis, DFMO and MGBG, given at concentrations of 3 mM and 36 μM, respectively, exerted strong inhibitory effects on Con A-induced proliferation of mouse T-lymphocytes (Fig. 1). The concentrations of inhibitors have been selected in our previous studies as effective but not cytotoxic [16].

Although the lymphocytes treated with inhibitors did not show any morphological changes under the light microscope, analysis of gene expression revealed marked changes in the expression of genes encoding cytoskeletal proteins as compared to cells not treated with inhibitor. In Con A-stimulated mouse T lymphocytes the level of β-actin mRNA increased during the first 3 h after Con A addition reaching maximal level 24–72 h later. The accumulation of α-tubulin mRNA occurred 24–72 h after Con A treatment (Fig. 2). The accumulation of both mRNA correlated with the timing of blast transformation and cell divisions.

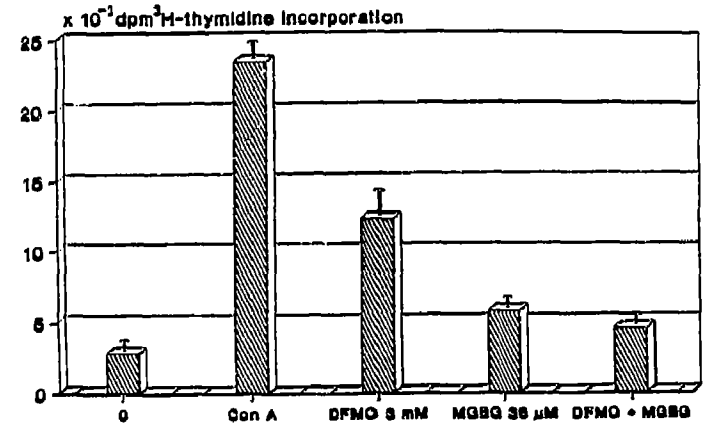

Fig. 1. The effects of inhibitors of polyamine biosynthesis on Con A-induced T lymphocyte proliferation. The rate of lymphocyte proliferation was measured by 6-h pulse labeling with [^{3}H]thymidine between 66 and 72 h of culture. The results are expressed as means ± S.D. The significance of the effects from repeated cultures (n = 30) calculated by Student's t-test was $P < 0.02$.

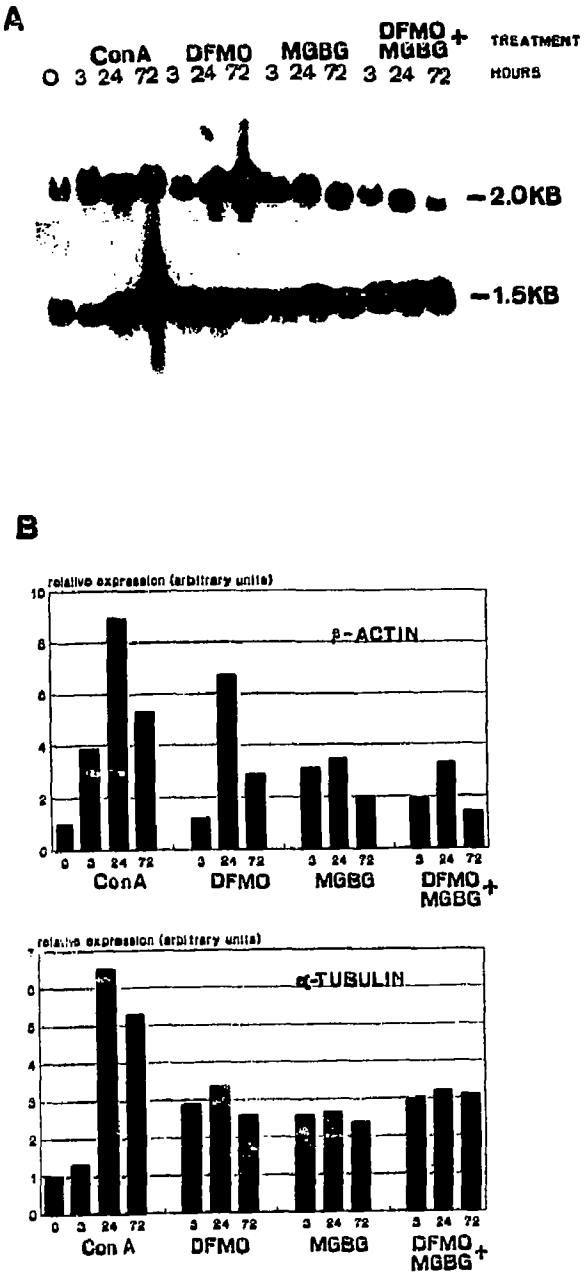

Fig. 2. The effects of inhibitors of polyamine biosynthesis on the expression of genes encoding cytoskeletal proteins. The top panel of the figure shows the expression of β-actin (2.0 kb) and α-tubulin (1.5 kb) in lymphocytes nontreated (○) and at different times after treatment with: Con A, 3 mM DFMO and/or 36 μM MGBG. The relative abundance of particular mRNAs based on the densitometric readings is presented on the lower panel (for details see section 2).

Both inhibitors, DFMO and MGBG as well as their combination, prevented accumulation of mRNAs encoding β-actin and α-tubulin (Fig. 2, top panel). Particularly strong was the inhibition of mRNAs accumulation occurring between 24 and 72 h after Con A addition, especially in the case of the effect of MGBG on the expression of β-actin. The inhibitory effect of MGBG on β-actin gene expression was seen very clearly even after the first 3 h following mitogenic stimulation (Fig. 2). Those results may provide an explanation for the data showing disruption of actin and tubulin networks reported by Pohjanpelto et al. [11].

In conclusion, we would like to say that polyamines' influence on the structure of cytoskeleton and, in consequence, on cell proliferation may be, at least in part, mediated through effects of polyamines on the expression of genes encoding major cytoskeletal proteins. The importance of this finding is further emphasized by the fact that it has been reported that several peaks of polyamine synthesis occur in the cell cycle; the first one after a few hours after mitogenic stimulation, and others preceding DNA synthesis and cell division [4,10]. Therefore, one may expect that depletion of polyamines may impair several different components of the cell cycle, namely growth in size, DNA replication and cell division, as all these are dependent on the integrity of the cytoskeletal structure which in turn depends on polyamines. For instance, it is well known that actin and tubulin are important components of, respectively, the contractible ring and mitotic spindle, the integrity of which is critical for normal cell division.

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