Bull Environ Contam Toxicol (2009) 83:15–18
DOI 10.1007/s00128-009-9709-5

Dimethylarsinous Acid Disturbs Cytokinesis

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Received: 7 April 2008 / Accepted: 16 March 2009 / Published online: 25 March 2009

Abstract Dimethylarsinous acid (DMAIII) induced tetraploidy and multinucleation in V79 cells. While the highest yield of tetraploids and multinucleated cells was at 1.25 μM of DMAIII, the mitotic index was highest at DMAIII 2.5 μM due to mitotic arrest. Mitosis was not observed at 5 μM. We observed V79 cells treated with DMAIII and visualized with rhodamine-phalloidin. Abnormal actin location was observed in the dividing cells which were treated with DMAIII and visualized with rhodamine-phalloidin. These findings suggested strongly that DMAIII inhibits not only formation of the normal mitotic spindle but cytokinesis and induces the formation of multinuclear cells.

Keywords Dimethylarsinous acid · Cytokinesis · Multinucleus · Actin

It has been generally believed that methylation of arsenic can be considered a mechanism of detoxification. However, trivalent methylated arsenicals such as dimethylarsinous acid (DMAIII) and monomethylarsonous acid (MMAIII) are more toxic than the pentavalent forms (Aposhian et al. 1999). MMAIII and DMAIII are detected in the urine of individuals who have ingested inorganic arsenic-polluted drinking water over a long period. DMAIII disturbs the cell cycle and induces aneuploids and tetraploids (Kuroda et al. 2005). Ochi et al. (2003) found that DMAIII induces tetraploids and multinucleated cells and also induces multipolar spindles and multipolar division. They concluded that abnormal mitosis causes the multinucleated cells.

Liver tetraploidization is controlled by a new process (prevention of cleavage plane specification) of incomplete cytokinesis (Ducos et al. 2007). KLP3A, a presumptive motor protein, is a critical component in the establishment or stabilization of the central spindle. Furthermore, the central spindle is the source of signals that initiate the cleavage furrow in higher cells (Williams et al. 1995). Disruption of the actin cytoskeleton with cytochalasin D also eliminated the central spindle (Giansanti et al. 1997). From their results, Straight and Field (2000) suggest that the central spindle affect the structure of the contractile ring and the contractile ring can influence the structure of the central spindle, reversely. Multinucleated cells and tetraploids may not be caused by only abnormal mitosis.

To improve understanding of the induction of multinucleated cells and tetraploids with DMAIII, we examined the effects of DMAIII on actin filament assembly using V79 cells.

Materials and Methods

Iododimethylarsine for standard solution of DMAIII was obtained from Dr. W.R. Cullen (University of British Columbia, Vancouver, Canada). Iododimethylarsine in water is hydrolyzed to DMAIII. V79 cells, which originated from Chinese hamster lung, were obtained from the Institute for Fermentation (Osaka, Japan). Leibovitz-15

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(L-15) medium was purchased from Sigma-Aldrich, Japan. Fetal bovine serum was obtained from ICN Biochemicals (Costa Mesa, CA). Giemsa stain solution was obtained from Merck (Darmstadt, Germany). Trypsin was purchased from Difco (Michigan, USA). Other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan). V79 cells were cultured following the method of Kuroda et al. (2005).

Chromosome preparation was carried out as follows: the cells were given freshly dissolved DMAIII and kept for 8 h, followed by colcemid treatment (0.1 μg/mL) for 2 h, after which they were treated with a hypotonic solution of 0.075 M KCl and fixed with methanol–acetic acid (3:1). Metaphase figures were then stained with 2% Giemsa stain solution for 15 min. Metaphase figures with 38–50 chromosomes were regarded as tetraploids. The mitotic index (MI) was determined from the chromosome preparation without colcemid treatment as the proportion of cells with mitotic figures among 1,000 mononuclear cells.

The preparation of dividing cells for rhodamine-phalloidin staining was carried out as follows: the preculture cells were given DMAIII and kept for 2 h, after which dividing cells were separated from the flask by shaking, collected by centrifugation, and fixed with 0.1% formaldehyde. These cells were then washed twice with PBS and treated with 0.1% Triton X-100 for 3–5 min, following which actin filaments were stained with rhodamine-phalloidin including 1% BSA for 20 min. The cells were then again washed twice with PBS and stained with 4,6-diamidino-2-phenylindole (DAPI II 125 ng/mL, Vysis). After sealing with Perma Fluor Aqueous Mounting Medium (IMMUNON), the stained cells were observed by laser microscope (BX50, OLYMPUS).

Results and Discussion

The incidence rates of tetraploidy and multinucleation in the V79 cells were less than 1% each. With addition of DMAIII, induction of tetraploids and multinucleated cells was enhanced. The incidence rates of tetraploidy and multinucleation following exposure to DMAIII 1.25 μM were 62% and 61.4%, respectively, and decreased to 6.5% and 12.3%, respectively, at DMAIII 2.5 μM. At DMAIII 5.0 μM, most of the cells seemed to be nonviable and the incidence rate was close to 0% (Fig. 1). DMAIII arrested the mitosis of V79 cells so strongly at DMAIII 5.0 μM that most of the mitotic cells were killed and the incidence of tetraploids was reduced.

The induction of tetraploids and multinucleated cells by exposure to DMAIII showed very similar patterns. The incidence of tetraploid cells showed a linear correlation with that of multinucleated cells ($r = 0.977$) (Fig. 2). This

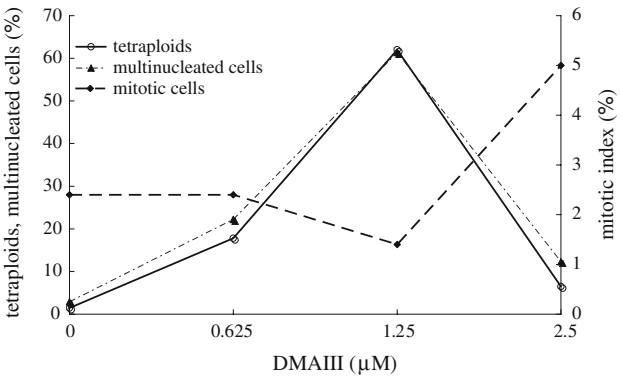

Fig. 1 Effects of DMAIII on V79 cells. The incidence rates of tetraploids and multinucleated cell in the V79 cells were less than 1% each. The incidence rates of tetraploids and multinucleated cell following exposure to DMAIII 1.25 μM were 62% and 61.4%, respectively, and decreased to 6.5% and 12.3%, respectively, at DMAIII 2.5 μM. At DMAIII 5.0 μM was down to 0%. The MI was 2.4% at DMAIII 0 and 0.625 μM and 1.4% at DMAIII 1.25 μM. Although the index increased to 5% at DMAIII 2.5 μM, it fell to 0% at DMAIII 5.0 μM

findings suggest that the mechanism of multinucleated cells and tetraploids may be the same.

The mitotic index was 2.4% at DMAIII 0 and 0.625 μM and 1.4% at DMAIII 1.25 μM. Although the index increased to 5% at DMAIII 2.5 μM, it fell to 0% at DMAIII 5.0 μM (Fig. 1). The increase in the mitotic index was not due to enhancement of mitosis but to mitotic arrest since DMAIII is known as a strong spindle poisons (Ochi et al. 2003; Kuroda et al. 2005).

Tetraploids are also usually induced by spindle poisons such as colchicine (Matsuoka et al. 1997). In this study, the pyramid tip of mitotic arrest did not coincide with that of tetraplodization. Premature chromosome condensation (PPC), which occurs when one nucleus in a di-nucleate enters the M-phase while the other is not synchronized, displayed a level of about 5% at DMAIII 1.25 μM (data

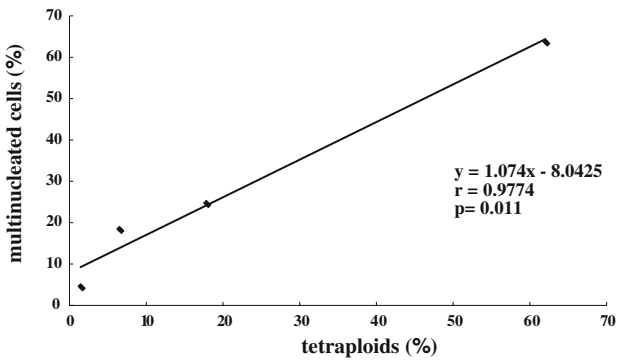

Fig. 2 Relationship between tetraploids (%) and multinucleated cells (%) induced by DMAIII in V79 cells. The incidence of tetraploids showed a linear correlation with that of multinucleated cells ($r = 0.977$)

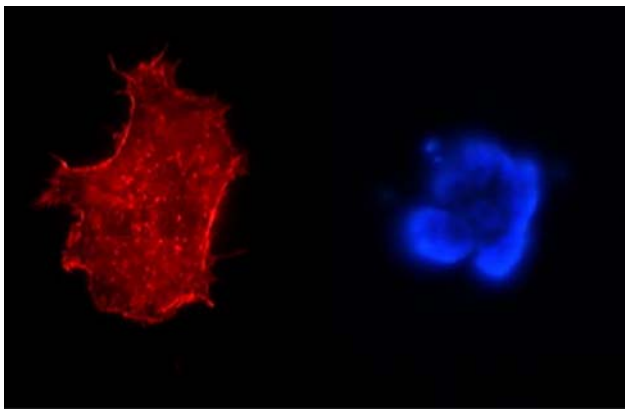

Fig. 3 Control cells stained with rhodamine-phalloidin (*left*) and DAPI (*right*) in interphase. Note the variety of filamentous structures that extended throughout the cell, actin filaments from many types of cell-surface projections (*left*). The chromosomes are extended and much of their chromatin exists as long, thin tangle threads in the nucleus so that individual chromosomes cannot be easily distinguished (*right*)

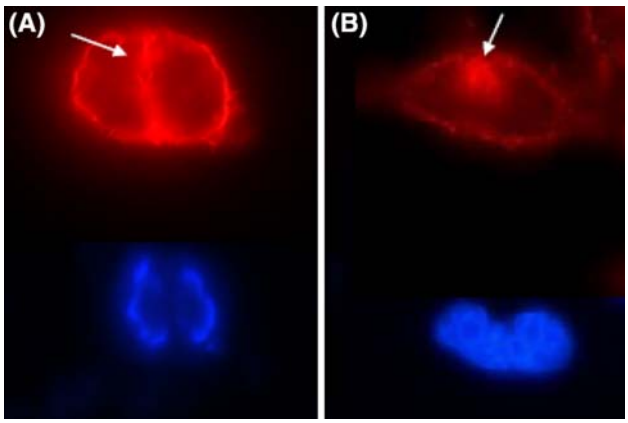

Fig. 4 **a** Control cells stained with rhodamine-phalloidin (*upper section*) and DAPI (*lower section*) in telophase. Inside the cells, we can see the contractile ring (*arrow*) assembles transiently to divide cells into two during cytokinesis (*upper section*). The sister chromatids synchronously separate to form two daughter chromosomes (*lower section*). **b** DMAIII-treated cells with staining with rhodamine-phalloidin (*upper section*) and DAPI (*lower section*) in telophase. Abnormal actin localization was detected (*arrow*); it was not started contractile ring form (*upper section*). The sister chromatids synchronously started to separate to form two daughter chromosomes (*lower section*)

were not shown). If the cell cycle of the two nuclei in a di-nucleate is synchronized, the figure of the metaphase is observed as tetraploids. Most of the tetraploids induced by DMAIII may display a metaphase figure in which the two nuclei in the di-nucleate enter the M phase synchronously. These findings suggest that multinucleated cells and tetraploids may be induced not by mitotic arrest but by the inhibition of cytokinesis.

The effects of DMAIII on cytokinesis were observed by actin staining. Control cells in the interphase are shown in Fig. 3 (left: stained with rhodamine-phalloidin; right: stained with DAPI): the actin filaments form many types of cell-surface projection and the chromosomes are extended. In control cells, the actin filaments formed either transient or stable structures; the contractile ring assembled transiently to divide the cell into two during cytokinesis and nuclei stained with DAPI were separating to form two daughter chromosomes (Fig. 4a). As shown in Fig. 4b, abnormal actin localization was observed in the DMAIII 1.25 μM-treated cells stained with rhodamine-phalloidin in the telophase; two nuclei were formed in the cell but the assembly of actin was not localized in the center of the cell. These results show that DMAIII inhibited normal actin assembly and induced di-nucleated cells. In this experiment, DMAIII induces giant clots of actin filaments near the plasma membrane in the cells (Fig. 4b). Cytokinesis interfered by DMAIII was similar with psychosine (galactosylshingosine). It forms giant clots of actin filaments in the mitotic phase and induces di-nucleation (Kanazawa et al. 2000).

An experiment using nocodazole suggests that the microtubule play an important role in the formation of the contractile ring (Takayama et al. 2002).

DMAIII is considered as a microtubule inhibitor, since it induces mitotic arrest as demonstrated in the present experiment and previous works (Ochi et al. 2003; Kuroda et al. 2005). It may be a possible mechanism that DMAIII disturbs cytokinesis by abnormal spindle formation. But the peak concentration of DMAIII inducing mitotic arrest was different from that inducing tetraploids and multinucleated cells. DMAIII might affect independently tubulin assembly and actin filament assembly.

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