MYXOTHIAZOL: A REVERSIBLE BLOCKER OF THE CELL CYCLE

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Myxothiazol, a potent inhibitor of the cytochrome bc_1 oxidoreductase, was shown by the use of flow cytometry to block reversibly the late G_1/S phase of the cell cycle of human lymphoblastic T-cell line Jurkat (clone 886) at concentrations of 0.5 μg/ml. These observations are compared to those of other drugs, such as antimycin, which effect the respiratory chain, and with O_2-deficiency.

Myxothiazol, an antibiotic isolated from myxobacteria[1~4] has been established as a potent inhibitor of the mitochondrial bc_1 complex distinct from antimycin[5~12] and other inhibitors of cellular respiration[13]. Mammalian cell lines with resistance to antimycin encoded by the mitochondrial cytochrome b gene do not show any cross-resistance to myxothiazol[14,15]. As cellular respiratory metabolism has a direct effect on the cell cycle clock[16], O_2-deficiency or drugs acting on cellular respiration may have different effects on the cell cycle[17~20]. We have therefore investigated the action of myxothiazol on the cell cycle of the human lymphoblastic T-cell line Jurkat (clone 886). In experiments using flow cytometry with the acridine orange staining technique[21] differences in the arrested state between cells treated with myxothiazol and those treated with standard blocking regimens were observed.

Materials and Methods

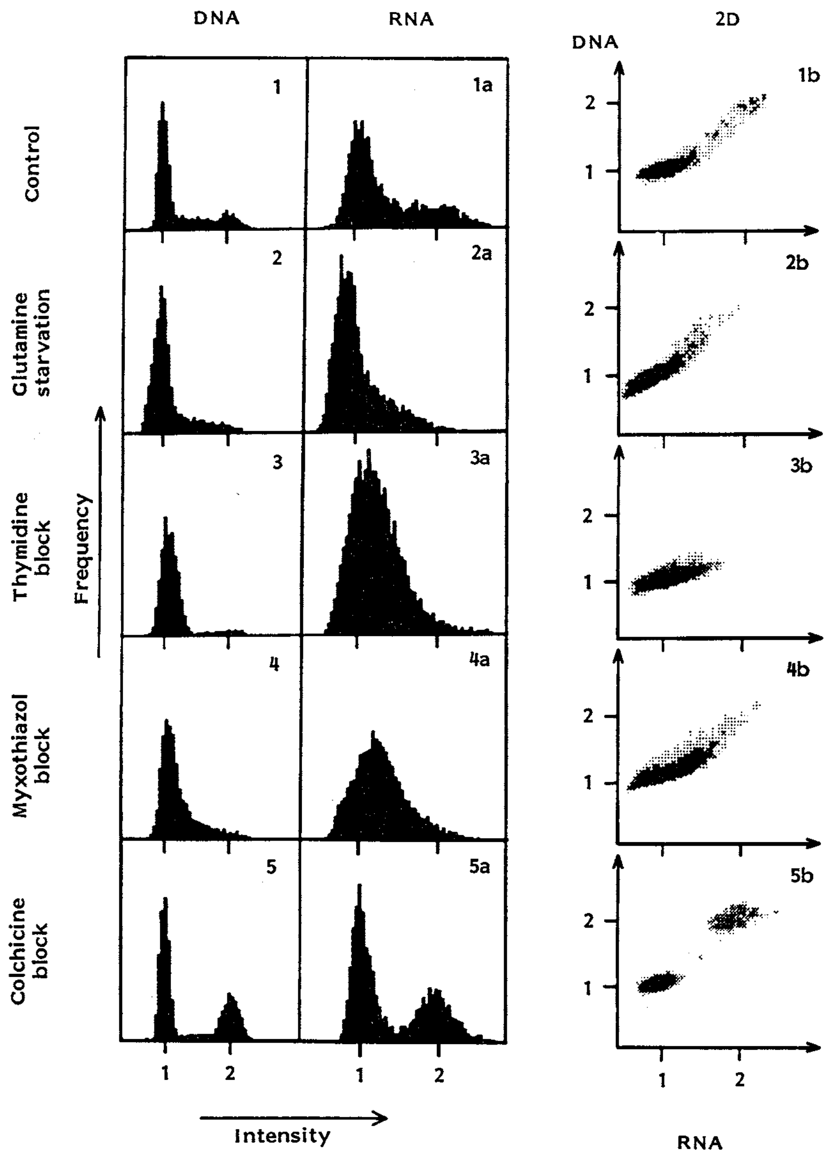
Cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS). Jurkat (clone 886) was isolated by cloning cells in soft agar. Viable cell counts were determined by Trypan blue exclusion. For cell cycle studies stationary cells were harvested, seeded at 4×10^5 cells per ml in fresh medium. After 24 hours the various blockers were added or the cells were seeded in starvation medium for 24 to 48 hours (for details see legends of Figs. 1 and 2). The cells were released from the block by washing twice and seeding in fresh medium. For cytometric analysis the cells were harvested by centrifugation, suspended in phosphate buffered saline (PBS; 20 mM K-phosphate, 150 mM NaCl pH 7.2) at 5×10^6 cells per ml, and fixed by addition of 9 volumes of ethanol and stored at $-20°C$. For staining 5×10^5 cells were resuspended in 0.75 ml PBS and 1.8 ml acridine orange staining solution pH 6.0 (0.2 M Na_2HPO_4, 0.1 M citric acid, 1 mM EDTA, 0.15 M NaCl and 12 μg/ml acridine orange)[22] was added. Cells were analyzed in an EPICS-C Flow cytometer (Coulter Electronics) with 300 mW laserpower at the argon 488 nm line. HV-settings of the photomultipliers were standardized with fluorescent full-bright beads (Coulter Electronics). Green fluorescence (515~570 nm) and red fluorescence (above 610 nm) signals were collected from 10,000 events routinely gated on the viable cell peak in the log forward angle light scatter.

Results

Initially we compared the action of myxothiazol on the cell cycle of Jurkat (clone 886) cells with

† Deceased.

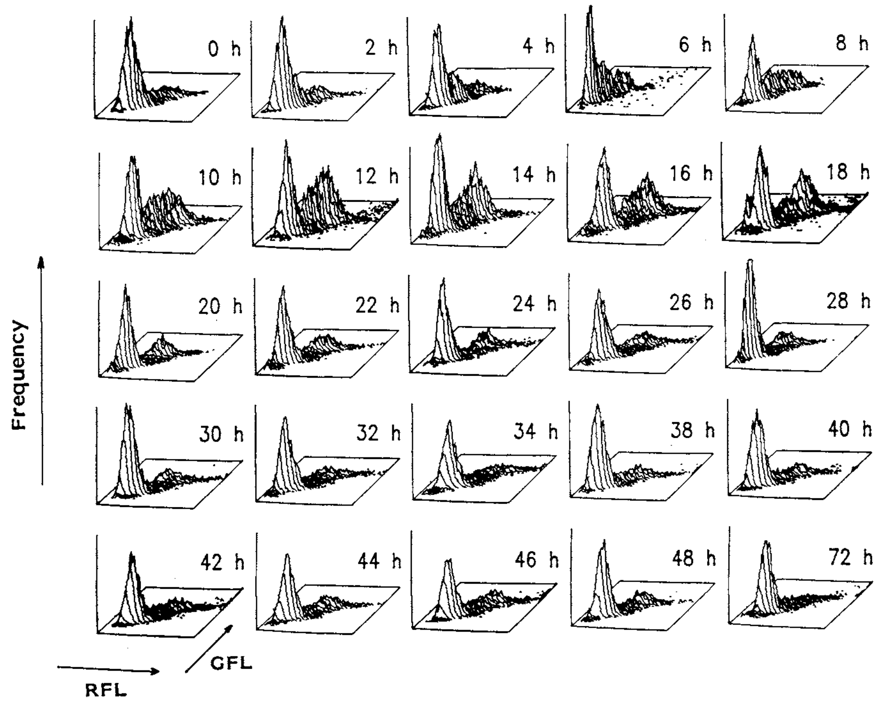
Fig. 1. Characterization of myxothiazol block.



DNA and RNA histograms and correlated 2D plots as measured by flow cytometry.

standard inhibitors of known mechanism (Fig. 1). Typical distributions of DNA and RNA, as well as correlated 2D diagrams of growing Jurkat cells and those treated with various inhibitors, are shown in Fig. 1. As we were particularly interested in mapping the G_1 subcompartment of arrest exactly, the acridine orange technique was used; this allows G_1 cells to be subdivided into G_{1a} and G_{1b} by their differences in RNA content and inability of G_{1a} cells to enter directly the S phase[23,24]. Comparing normal medium (histogram 1) with glutamine starvation (histogram 2) most of the green fluorescence of the latter was concentrated in the peak of 2cDNA content whereas the 4cDNA compartment was depleted; S phase cells were also found less frequently. Similiarly, the content of RNA per cell was reduced in cells accumulating in G_1 (histogram 2a), indicating the cells were arrested in G_{1a}. Cells

Fig. 2. Cell cycle kinetics after release from myxothiazol block.



DNA and RNA frequency histograms measured by flow cytometry (3-dimensional plots). Stationary cells were harvested, treated for 24 hours with 0.5 μg/ml myxothiazol in RPMI 1640 containing 10% FCS. The cells were washed with fresh medium and seeded with 4×10^5 cells per ml. After release from the block (time 0 h) the cells were monitored over a period of 72 hours (two complete cell cycles).

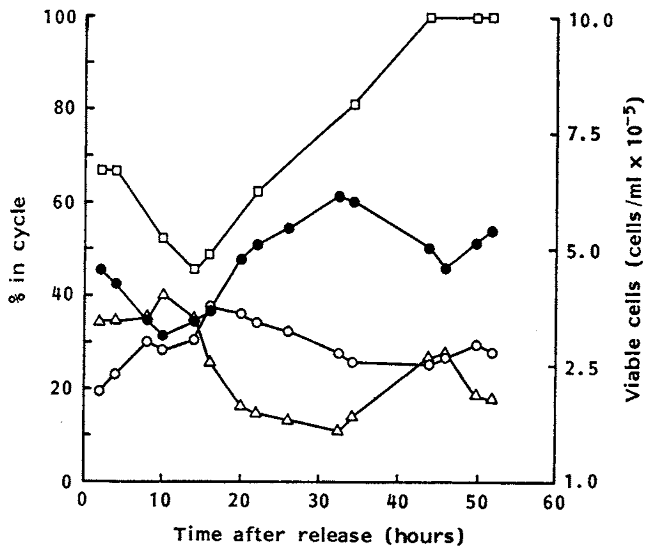
GFL: Green fluorescence, RFL: red fluorescence.

treated with 1 μg/ml myxothiazol (histogram 4) show a similiar DNA distribution with a reduced S phase and with no cell division. In contrast, the RNA content is significantly higher for the G_1 phase (histogram 4a) than for cells under glutamine starvation, causing the cells to arrest in a very late G_1 phase (G_{1b}) extending into the S phase. A typical block at the G_{1b}/S transition as shown by thymidine, which acts on the deoxyribonucleotide reductase, is shown in Fig. 1 (histogram 3). The block is characterized by G_1 cells with high RNA content. Furthermore this treatment, in contrast to myxothiazol, results in only a few cells being in the DNA synthesis-phase. A particularly well defined G_1 peak, reduced S and elevated G_2/M peaks resulted from the exposure to colchicine (Fig. 1 histogram 5).

In order to exclude toxic effects the reversibility of the myxothiazol block was tested. Cells arrested with 0.5 μg/ml were washed and supplied with new medium. As can be seen from the data shown in Fig. 2 under these conditions the cells resumed their growth cycle. The cells reach a maximum of DNA synthesis activity after 12 hours and arrived at the G_2/M phase 6 hours later. Synchronization levels off after 2 cycles, hence the second wave of synthetic activity is not readily seen in the 3-dimensional plots. When percentages of cell cycle compartments are calculated and plotted (Fig. 3) a cycle time of 34 hours is derived. This fits well with the cell cycle time for the clone Jurkat (clone 886) determined from the logarithmic growth curve without addition of an inhibitor (data not shown). The degree of synchronization is similar to the behavior observed with thymidine and hydroxyurea blocks (data not shown).

Fig. 3. Distribution of cell cycle compartments of Jurkat clone 886 cells after release from myxothiazol block.

● % cells in G_0/G_1, ○ % cells in S, △ % cells in G_2/M, □ viable cells.



Discussion

The mode of action of myxothiazol on the respiratory chain has been investigated in detail at the mitochondrial level[14,15]. Effects on the cell cycle of mammalian cells have, to our knowledge, not been reported previously. It was shown that myxothiazol arrests cells of the human lymphoblastic T-cell line Jurkat in the late G_1/S phase which is different to the arrest in G_1 caused by a nutrient block. The S phase content places the phase later in the cycle than the G_1/S transition seen after thymidine exposure. The action of other inhibitors of the respiratory chain, such as rotenone, antimycin and O_2-deficiency on the cell cycle has been published for the Ehrlich ascites cell line[17~20]. Compared with these results, the action of myxothiazol is different to that of rotenone as the cells can be released from the block without later toxic effects and, in addition, the position of the block in the cycle is different. Electronmicroscopic studies of thin sections of myxothiazol-treated cells showed no significant ultrastructural changes of the mitochondria (H. LÜNSDORF and K. DITTMAR; unpublished results) in contrast to rotenone-treated cells[17]. The latter is also true for O_2-deficiency[18~20]. Antimycin on the other hand was shown to arrest cells in the G_1 phase whereas S and G_2-cells were capable of completing the cycle. A comparison of both antibiotics may reveal similarities in their mode of action as cell cycle inhibitors.

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