A macrolide antibiotic, roxithromycin, inhibits the growth of human myeloid leukemia HL60 cells by producing multinucleate cells

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Abstract

The antiproliferative effect of roxithromycin (RXM) was studied using human myeloid leukemia HL60 cells. RXM inhibited the growth of HL60 cells in a concentration-dependent manner, and significantly inhibited growth at concentrations above 75 µM. This growth inhibition was not associated with specific cell cycle arrest and DNA synthesis was not impaired. In addition, the number of viable cells remained almost unchanged in the presence of 100 µM RXM. RXM induced growth inhibition at least partly by the formation of multinucleate cells. Both flowcytometric and morphological examination revealed that more than 40% of the RXM-treated cells were binucleate. These findings demonstrate that RXM is a potent new modulator of cell cycle progression in HL60 cells and suggest that the inhibition of cytokinesis by this drug may provide a new model for studying mitosis. (Mol Cell Biochem 144: 191-195, 1995)

Key words: roxithromycin, macrolide antibiotics, myeloid leukemia, hyperploid, multinucleate cell, cytokinesis

Introduction

Macrolide antibiotics are not only used to treat Gram-positive organisms but are also given for non-bacterial infections such as Mycoplasma pneumoniae. In addition, these antibiotics are reported to directly activate anti-inflammatory cells [1, 2] and to alter the cytokine network [3, 4]. One of the macrolide antibiotics, roxithromycin (RXM), inhibits T-lymphocyte transformation induced by mitogen stimulation [5, 6], but its precise effect on the cell cycle remains unclear.

Cell proliferation is governed by the control mechanism of cell cycle progression. In general, progression through the cell cycle is sequential and no further stimulation is required to continue this process once a cell passes the restriction point in the G1 phase (see review [7]). Certain environmental changes have been shown to influence the cell cycle of cancer cells. For example, the induction of differentiation in leukemic cells by chemical inducers and cytokines is associated with cessation of growth [8-10]. Most growth inhibitors modulate the cell cycle at the G1 phase, but a few substances are known to affect the G2 and M phases. Trichostatin A, Leptomycin B, and staurosporine are the most intensively studied chemicals that modulate the G2 to M transition [11, 12], with all of these drugs inducing G2 arrest. In addition, trichostatin A and Leptomycin B also induce tetraploid cells after their removal from the culture medium [11, 12]. Staurosporine is a potent protein kinase C inhibitor that induces G2 arrest [13]. Unlike trichostatin A, however,
removal of staurosporine allows cells to enter into mitosis. This difference suggests that the mechanisms of action of these two drugs differ in detail. Cytochalasin B [14], phalloidin [15], myosin light-chain kinase inhibitors [16], exoenzyme C3 of Clostridium botulinum [17] and Bordetella bronchiseptica dermonecrotic toxin (DNT) [18] are other inhibitors of cytokinesis. In contrast to the G2 and M transition inhibitors, however, Bordetella bronchiseptica dermonecrotic toxin accelerates DNA synthesis [18].

The present study focused on the effect of RXM on the proliferation of human myeloid leukemic HL60 cells and demonstrated that RXM inhibited cell growth. This growth inhibition was not associated with the impairment of DNA synthesis but with inhibition of cytokinesis in association with the formation of multinucleate cells.

Materials and methods

Cell culture

Human promyelocytic leukemia HL60 cells were obtained from the Japanese Cancer Research Resources Bank. Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (Hyclone) containing 100 U/ml penicillin and 100 μg/ml streptomycin in a 5% CO2 incubator at 100% humidity. Exponentially growing cells were used for the experiment. RXM (a kind gift from Roussel Uclaf) was dissolved in phosphate buffer (pH 6.5) to give a 1 mM stock solution and was stored at -20°C until use. Cell viability was calculated by the trypan blue dye exclusion method or by a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Chemicon), as described previously [19].

Cell cycle analysis

The cellular DNA content was analyzed using a FACscan (Becton Dickinson) after propidium iodide staining. The percentage of cells in each phase of the cell cycle was calculated by the sum of broadened rectangles method.

Assessment of DNA synthesis

DNA synthesis by RXM-treated cells was studied using bromodeoxyuridine (BrdU) incorporation. Cells were treated with 10 μM BrdU (Sigma) for 30 min, harvested, and fixed in 70% ethanol. Incorporated BrdU was detected with an FITC-conjugated anti-BrdU antibody (Becton Dickinson) according to the manufacturer's directions. Treated cells were also stained with propidium iodide and the fluorescence intensity was determined by flow cytometry.

Morphological studies

HL60 cells, treated with 100 μM RXM for 48 h, were subjected to cytopsinning and were stained by May-Grünwald-Giemsa method. Then multinucleate cells were assessed by histological examination.

Results

Growth inhibition by RXM

The number of RXM-treated cells was calculated by the MTT and the trypan blue dye exclusion methods. The effect of RXM on cell growth was assessed after 72 h of incubation in the presence of various concentrations of the drug. As shown in Fig. 1, the growth of HL60 cells was inhibited in a concentration-dependent manner, with significant inhibitory activity being observed at RXM levels of 75 μM and higher levels (Fig. 1). Cell growth in the presence of 100 μM RXM was studied in detail (Fig. 2). A significant reduction of cell numbers compared with the control culture was first observed at 24 h. Since the substantial number of RXM-treated cells at 48 h remained unchanged from the initial number, it was about 25% of control cells. These findings demonstrate that RXM had a cytostatic effect at 100 μM.

Fig. 1. Growth inhibitory activity of RXM. Exponentially growing cells were treated with various concentrations of RXM or with buffer alone for control cells and harvested after 72 h. Cell growth was measured by the MTT assay, as described in the text. Significant growth inhibition was observed at a concentration of 75 μM or more. Each experiment was done in triplicate.