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Brequinar

Potentiation of the Antiproliferative Activity of Brequinar Sodium for Murine Lymphocytes by Exogenous Cytidine

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THE antimetabolite brequinar sodium (BQR) is a novel quinoline carboxylic acid analogue with broad antitumor activity in mice, including inhibition of the growth of tumor xenografts.[1] The mechanism of action of BQR in tumor cells is believed to involve inhibition of dihydroorotate dehydrogenase, a step in de novo pyrimidine biosynthesis,[2,3] resulting in depletion of precursors required for RNA and DNA synthesis. There is recent evidence that BQR is very effective either alone or in combination with FK 506 or cyclosporine (CyA) in the prolongation of experimental allograft or xenograft survival.[4] To obtain more information on the influence of BQR on T- and B-cell activation and proliferation, we have investigated its effects on the responses of murine spleen cells to various stimulants, including concanavalin A (ConA), phorbol myristate acetate (PMA) ± ionomycin, anti-CD3, and anti-Igs. Since BQR inhibits pyrimidine biosynthesis, we have also determined the effects of exogenous uridine and cytidine on the antilymphocytic activities of the drug in vitro.

MATERIALS AND METHODS

Reagents

BQR (DuPont Merck Pharmaceutical Co, Wilmington, Del) was kindly provided by Dr L. Makowka, Cedars-Sinai Medical Center (Los Angeles, Calif). It was dissolved fresh before each experiment in sterile saline (1 mg/mL) and diluted in RPMI-1640 (Gibco, Grand-Island, NY). ConA, PMA, ionomycin, uridine, cytidine, and goat anti-mouse Igs were from Sigma Chemical Co (St Louis, Mo). Antimurine CD3 monoclonal antibody (MAb; clone KT3) was from Serotec (Oxford, England, UK).

Lymphocyte Culture

Nucleated spleen cells isolated from spleens of 8- to 12-week-old, male C57BL/10 SnJ mice (Jackson Laboratory, Bar Harbor, Me) were prepared as described previously[5] in RPMI-1640 with 10% vol/vol fetal bovine serum (FBS) and 5×10^5 cells added to triplicate round-bottom wells of 96-well microtiter plates (Corning, NY). Cells were stimulated with ConA (0.5 to 5 μg/mL), PMA (1 ng/mL), ionomycin (0.5 μmol/L), anti-CD3 (1 of 100), or anti-Ig (1 of 50) for 24 to 120 hours. For estimation of DNA synthesis, 1 μCi ^{3}H-thymidine was added 24 hours before harvest and incorporation of radioactivity into DNA estimated by liquid scintillation counting.

Quantitation of Cytokine Production and Cytokine Gene Expression

Interleukin-2 (IL-2) and IL-4 levels in supernatants of cultures of 50×10^6 spleen cells stimulated for 20 hours with ConA (5 μg/mL) in Falcon tissue culture tubes were estimated using enzyme-linked immunosorbent assay (ELISA) kits for murine IL-2 (Collaborative Research Inc, Bedford, Mass) or IL-4 (Endogen Inc, Boston, Mass). Production of mRNA for IL-2 and IL-4 in ConA-stimulated cells was determined by semiquantitative reverse transcription polymerase chain reaction as described elsewhere.[6]

RESULTS AND DISCUSSION

In 72-hour cultures, addition of BQR (5 μg/mL to 0.1 μg/mL) at 0 hour caused dose-dependent inhibition of the strong proliferative responses induced by both ConA (5 μg/mL) (Fig 1a) and PMA + ionomycin (data not shown). In contrast, no impairment of low concentration ConA (0.5

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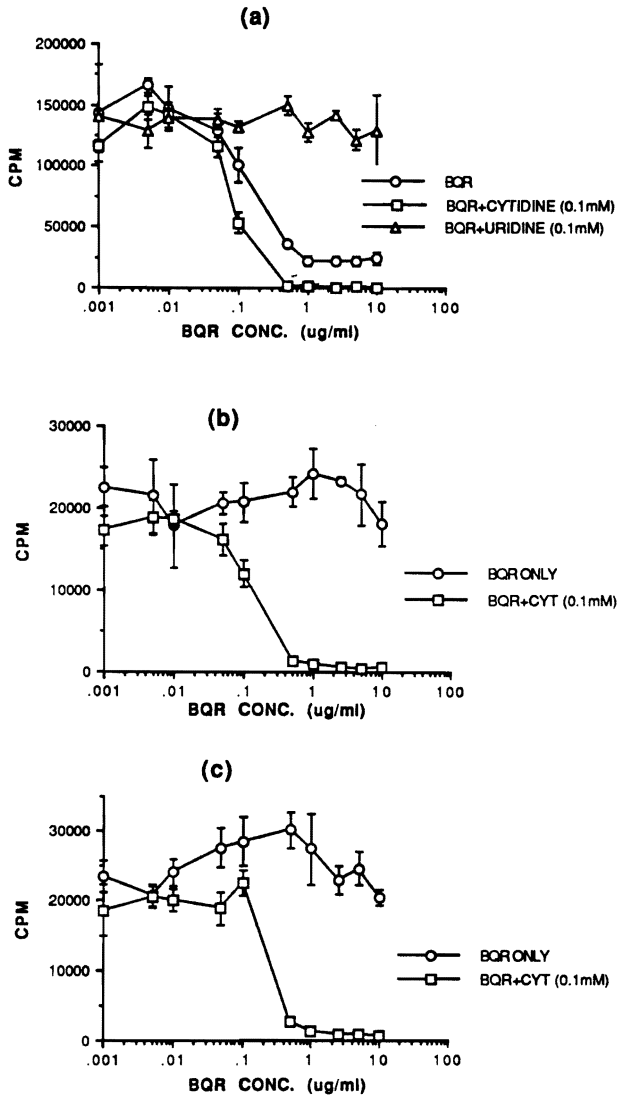

Fig 1. Effects of exogenous nucleosides on the antiproliferative activity of BQR. **(a)** The inhibitory effect of BQR on ConA (5 μg/mL)-stimulated DNA synthesis was reversed by uridine but potentiated by cytidine. **(b)** Inhibition of weaker anti-CD3–induced DNA synthesis by BQR + cytidine but not by BQR alone. **(c)** Inhibition of anti-Ig–induced DNA synthesis by BQR + cytidine but not by BQR alone.

or 0.1 μg/mL), anti-CD3, or anti-Ig responses was observed.

Addition of BQR to ConA (5 μg/mL) cultures as late as 24 hours gave 70% inhibition of DNA synthesis. The capacity of synthetic nucleosides to reverse the inhibitory effect of BQR was examined by addition of either uridine (0.1 mmol/L) or cytidine (0.1 mmol/L) together with BQR at the start of ConA-, anti-CD3–, or anti-Ig–stimulated cultures. Uridine almost totally reversed the inhibitory effect of BQR, whilst in contrast, cytidine potentiated its action (Fig 1a–c). Cytidine alone had a modest inhibitory effect on ConA-stimulated DNA synthesis. These observations are consistent with inhibition by BQR of a step in de novo pyrimidine nucleotide biosynthesis. Moreover, they suggest that ConA-stimulated murine lymphocytes may lack cytidine deaminase, which permits formation of uridine or its anabolites from cytidine (salvage pathway). ConA (5 μg/mL)-stimulated lymphocytes cultured for 24 hours in the presence of BQR (5 μg/mL) showed no inhibition of production of mRNA for IL-2 or IL-4 or of cytokine production.

Combination of BQR with cytidine inhibited IL-2 and IL-4 production but not mRNA expression (data not shown). The observed antimetabolic effects of BQR complement the cytokine synthesis inhibitory action of FK 506 or CyA and suggest that this new agent may prove useful as adjunctive immunosuppressive therapy in combination with either FK 506 or CyA. Moreover, combination of BQR and cytidine may offer a further possibility for inhibition of T- and B-cell proliferation.

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