The effect of the catalytic topoisomerase II inhibitor dexrazoxane (ICRF-187) on CC9C10 hybridoma viability and productivity

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Received 25 June 2001; accepted in revised form 5 March 2002

Key words: antibody, antibody production, caspase, CC9C10, dexrazoxane, hybridoma, monoclonal antibody, topoisomerase II

Abstract
The effect of dexrazoxane on monoclonal antibody (Mab) production by CC9C10 hybridoma cells was investigated. Dexrazoxane is a catalytic inhibitor of DNA topoisomerase II. DNA topoisomerase II has a critical role in DNA metabolism and its inhibition by dexrazoxane can prevent completion of cytokinesis. Incubation of hybridomas with dexrazoxane was found to increase specific monoclonal antibody production by up to four-fold. However, due to the growth inhibitory effects of dexrazoxane the total Mab yield decreased by 40%. Under high density culture conditions (defined here as 10⁶ cells ml⁻¹) specific monoclonal antibody production increased by up to 37%, which was, however, accompanied by up to a 48% decrease in Mab yield. Hybridomas that were incubated with dexrazoxane significantly increased in size due to the inhibition of cytokinesis. Dexrazoxane was also observed to induce a delayed apoptosis in the hybridomas. The caspase inhibitor Z-V-AD-fmk slightly decreased the apoptotic effects of dexrazoxane. Preincubation with the caspase inhibitor Z-Asp-CH₂-DCB had no effect on dexrazoxane-treated hybridomas, but it did have antiapoptotic effects on the untreated hybridomas which normally undergo a significant basal level of apoptosis. In conclusion, dexrazoxane-induced growth inhibition (which results in higher specific antibody production) and apoptosis inhibition (which results in prolonged viability) has the potential to significantly enhance the productivity of hybridoma cell cultures.

Abbreviations: DMEM, Dulbecco’s Modified Eagle’s Medium; ELISA, enzyme-linked immunosorbent assay; HEPES, N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid]; Mab, monoclonal antibody; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; PBS, phosphate-buffered saline (145 mM NaCl, 10.7 mM Na₂HPO₄, 2.5 mM NaH₂PO₄, pH 7.2); qMab, specific monoclonal antibody production (µg antibody (10⁶ cells)⁻¹ d⁻¹); SFM, serum-free medium; Tᵢ, total cell index, integral of total cell density and time; TE, 10 mM Tris-HCl, 1 mM disodium EDTA buffer, pH 8.0; TBE, 100 mM Tris-base, 100 mM boric acid, 20 mM disodium EDTA, 0.2 µg ml⁻¹ ethidium bromide buffer, pH 8.3; Vᵢ, viability index, integral of viable cell density and time; Z-Asp, benzylloxycarbonyl-Asp-2,6-dichlorobenzoyloxymethylketone or Z-Asp-CH₂-DCB; Z-V-AD, benzylloxycarbonyl-Val-Ala-Asp-fluoromethylketone or Z-V-AD-fmk.

Introduction
One of the goals of monoclonal antibody (Mab) production technology is the development of processes aimed at improving cell culture efficiency. To this end, conditions that favor increased specific Mab productivity may be beneficial and cost effective. Various reports have indicated that high antibody production can occur under conditions that are not optimal for hybridoma cell growth. Culture conditions of hyperosmolarity, high temperature or addition of growth inhibitors have been shown to increase the specific...
Mab production rates (Barnabé and Butler, 1994; Ozturk and Palsson, 1991; Whiteside et al., 1992). This suggests the possibility of dissociating the conditions optimal for cell growth from the conditions optimal for monoclonal antibody production.

Hybridoma cells are, however, prone to apoptosis which is also a critical factor limiting the productivity of cultures. This is due to the relation between the number of viable cells and Mab production (Renard et al., 1988). Apoptosis or programmed cell death is characterized by distinct biochemical and morphological changes such as activation of caspases and nucleosomal DNA fragmentation (Nicholson and Thornberry, 1997; Wyllie, 1980). Apoptosis in hybridomas may be induced by nutrient depletion or by the addition of growth inhibitors (Cotter et al., 1992; Mercille and Massie, 1994). An ideal growth inhibitor would arrest cell growth without inducing apoptosis.

Dexrazoxane (Figure 1A) is used clinically to reduce doxorubicin-induced cardiotoxicity (Hasinoff, 1998; Hasinoff et al., 1998a). Under physiological conditions dexrazoxane undergoes a slow ring-opening hydrolysis to ADR-925 (Figure 1A) (Hasinoff, 1998; Hasinoff et al., 1998a), an analog of EDTA. Dexrazoxane likely exerts its cardioprotective effects through its rings-opened hydrolysis product ADR-925 by virtue of its ability to strongly chelate free iron, or to quickly and efficiently remove iron from its complex with doxorubicin (Hasinoff, 1998; Hasinoff et al., 1998a), thus reducing doxorubicin-induced iron-based oxygen free radical damage.

The bisdioxopiperazine dexrazoxane (ICRF-187, Zinecard®) is also a strong catalytic inhibitor of mammalian DNA topoisomerase II (EC 5.99.1.3) (Fortune and Osheroff, 2000; Hasinoff et al., 1995; Ishida et al., 1991). Dexrazoxane has been shown not to induce DNA-topoisomerase II covalent complexes, unlike the topoisomerase II poisons, etoposide and doxorubicin (Fortune and Osheroff, 2000; Ishida et al., 1991). Topoisomerase II alters DNA topology by catalyzing the passing of an intact DNA double helix through a transient double-stranded break made in a second helix (Fortune and Osheroff, 2000). Topoisomerase II has a critical role in DNA metabolism including replication, transcription and recombination and is required for the separation of chromosomes during mitosis. The bisdioxopiperazines have been proposed to act by trapping the enzyme in the form of a closed ATP-modulated protein clamp (Roca et al., 1994), thus preventing the formation or stabilization of cleavable complexes. Dexrazoxane may be promoting an energy-dependent inappropriate binding of topoisomerase II to DNA after the resealing step.

We previously showed (Hasinoff et al., 2000) that CHO cells that were continuously exposed to dexrazoxane did not complete cytokinesis, yet continued to increase in size (up to 150-fold greater volume) and ploidy (up to 32N DNA). We also showed that human leukemia K562 cells continuously exposed to dexrazoxane also dramatically increased in size and ploidy and also induced erythroid differentiation and a slow induction of apoptosis (Hasinoff et al., 2001). Given the fact that dexrazoxane was able to inhibit cell division, yet allowed the cells to continue to grow, we hypothesized that dexrazoxane might be able maximize antibody production in a monoclonal hybridoma cell line. In this study, we report how the growth inhibitory effects of dexrazoxane may alter the specific monoclonal antibody productivity of a well characterized mouse hybridoma cell line and how apoptotic induction may be prevented by caspase inhibitors.

**Materials and methods**

**Materials**

Dexrazoxane (Zinecard®, ICRF-187) was a gift from Pharmacia and Upjohn (Columbus, OH). Ultrapure agarose, DMEM, Ham’s F12 and Nunc Maxisorp ELISA plates were obtained from Gibco BRL (Burlington, ON). Cell Titer 96® Aqueous One Solution Cell Proliferation Assay was from Promega (Madison, WI). Z-VAD-fmk was from Alexis Biochemicals (San Diego, CA), and Z-Asp from Bachem (Torrance, CA). Chemicals not listed above were obtained from the Sigma Chemical Co. (St. Louis, MO) unless otherwise noted.

**Cells, media and incubation conditions**

CC9C10 hybridoma cells were obtained from the American Type Culture Collection (Rockville, MD). CC9C10 is a murine hybridoma cell which produces an antibody (IgG1) directed against bovine insulin. Cells were grown in a humidified atmosphere of 10% CO2 at 37 °C in a defined SFM buffered with 20 mM HEPES, pH 7.1 (Barnabé and Butler, 1994). Cells were counted with a hemocytometer and viability was determined by the trypan blue exclusion method.