SULPHONAMIDE INHIBITORS OF CARBONIC ANHYDRASE INHIBIT THE GROWTH OF HUMAN LYMPHOMA CELLS IN CULTURE

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ABSTRACT

Methazolamide and ethoxzolamide, which are very potent and specific inhibitors of carbonic anhydrase, strongly inhibited the growth of cells in culture, of a line established from a human lymphoma. Inhibition was achieved with concentrations of inhibitors in the micromolar range. Methazolamide inhibition was consistent over a range of cell concentrations, was maintained over a 6-day period and was dependent on inhibitor concentration.

It is suggested that inhibition of carbonic anhydrase may reduce provision of bicarbonate for nucleotide synthesis, especially as substrate for carbamoyl phosphate synthetase II, a key enzyme in the control of pyrimidine synthesis. The data are discussed in relation to cancer therapy.

**Keywords:** carbonic anhydrase, sulphonamide inhibition, nucleotide synthesis, carbamoyl phosphate synthetase, cancer therapy

INTRODUCTION
The zinc metalloenzyme carbonic anhydrase (CA; EC 4.2.1.1) catalyses the reversible hydration of CO2 (CO2 + H2O ⇌ HCO3− + H+). The enzyme appears to be ubiquitous in nature and is expressed in mammals in at least seven isozyme forms. CA I, II, III and VII are cytosolic, CA IV is membrane associated, CA V is mitochondrial and CA VI is secreted. CA II, which is among the most active of these enzymes, is expressed in virtually all tissue types. (For review, see [1].)

A common characteristic of all these isozymes is that they are strongly and, it would appear, specifically inhibited by certain aromatic and heterocyclic sulphonamides. Both human CA I and CA II are potently inhibited by methazolamide and ethoxzolamide having identical $K_i$ values of 0.01 μmol/L towards the former inhibitor and 0.002 μmol/L towards the latter [2].

A considerable body of evidence indicates that carbonic anhydrase is important for the provision of bicarbonate as the true substrate for early carboxylation steps in a number of biosynthetic processes. The use of sulphonamide inhibitors, at both cell and whole organ level, suggests a role for the enzyme in gluconeogenesis [3], ureogenesis [4] and lipogenesis [5,6] in several different animal species. It may well be that a low flux of HCO3− through these pathways may be accommodated by the uncatalysed rate of
bicarbonate provision, whilst metabolic conditions demanding a higher level of flux require the participation of carbonic anhydrase activity [7].

One such situation occurs in carcinoma cells where the enhanced rate of cell replication calls for a higher level of nucleotide synthesis than that required normally. Bicarbonate, not CO₂, is the substrate for carbamoyl phosphate synthetase II (CPS II), the glutamine utilizing, cytoplasmic isozyme which provides carbamoyl phosphate for de novo pyrimidine nucleotide synthesis (as opposed to the ammonia utilizing, mitochondrial CPS I isozyme which provides carbamoyl phosphate for the urea cycle) [8].

Similarly, bicarbonate is the substrate for the pyruvate carboxylase reaction [9], an early step in the production of glycine and glutamine, which, in turn, serve as substrates for purine nucleotide synthesis [10].

Whether bicarbonate is the substrate for the carboxylation step in the pathway of de novo purine synthesis is the subject of some debate, but seems to be species dependent. Whilst bicarbonate is the true substrate in *Escherichia coli*, there is some evidence that CO₂ may be used directly in chicken, although the reaction mechanism remains unclear [11,12].

Overall, it appears likely that carbonic anhydrase activity may be essential for the enhanced nucleotide synthesis evident in carcinoma cells and that specific inhibition of this enzyme may well be of therapeutic potential. In these studies, we therefore investigated the potential of sulphonamide CA inhibitors to block growth of tumour cells by virtue of their inhibitory action on nucleotide synthesis.

MATERIALS AND METHODS

**Media and reagents**

RPMI 1640 modified medium, which contained 20 mmol/L HEPES buffer but no sodium bicarbonate, was purchased from the Sigma Chemical Co., Poole, Dorset, UK. Fetal calf serum was purchased from Gibco Life Technologies Ltd., Renfrew, Scotland. In certain experiments, it was inactivated by heating at 56°C for 30 min. Tritiated thymidine was purchased from ICN Biomedicals Ltd., Thame, Oxfordshire, UK. Its specific activity was 64 Ci/mmol. The scintillation cocktail Optiphase Hisafe 2 was supplied by Wallac Scintillation Products, Turku, Finland. Sterile plastic ware was purchased from Bibby Sterilin Ltd., Stone, Staffordshire, UK. All other reagents were purchased from Sigma Chemical Co., Poole, Dorset, UK and were analytical grade where available.

**Cell line**

The premonocytic U937 cells were supplied by the European Collection of Animal Cell Cultures, PHLS, Porton Down, Wiltshire, UK. This cell line was established from malignant cells from the pleural effusion of a 37-year-old Caucasian male with diffuse histiocytic lymphoma.