

SHORT COMMUNICATION

Enhanced anti-proliferative action of busulphan by quercetin on the human leukaemia cell line K562

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The alkylating agent busulphan is commonly used as a single agent for the treatment of chronic myeloid leukaemia (CML). Patients receiving busulphan treatment for CML ultimately relapse since the malignant clone is not eradicated. Alternative treatments are clearly needed (Koller & Miller, 1986).

Combining two active drugs with different modes of action can sometimes result in enhanced therapeutic efficacy (Chang *et al.*, 1979). Analysis of data obtained from experiments to study this effect has been greatly facilitated with the computer program developed by Chou & Chou (1987), which is based on the median effect plot and multiple drug equation derived by Chou & Talalay (1984).

A recent report showed that quercetin enhanced the anti-proliferative activity of the alkylating agent nitrogen mustard (Hofmann *et al.*, 1988). Quercetin is a naturally occurring flavonoid which inhibits a wide range of enzymes including protein kinase C (PKC) (Gschwendt *et al.*, 1983), the tyrosine kinase encoded by the src oncogene (Graziani *et al.*, 1983), and other tyrosine kinases (Srivastava & Chiasson, 1986). PKC and several tyrosine kinases are located in or near the cell membrane and are involved in the transduction of growth factor signals to the nucleus. These enzymes therefore represent novel therapeutic targets for anti-cancer agents. Cells from patients with CML express an abnormal tyrosine kinase (Maxwell *et al.*, 1987) and the cells also contain high levels of PKC (Helfman *et al.*, 1983). We have therefore investigated the effect of combining the protein kinase inhibitor quercetin with busulphan on the proliferation of the CML-derived cell line K562 (Lozzio & Lozzio, 1975).

K562 cells were grown at 37°C in RPMI 1640 medium containing fetal calf serum (10%), glutamine (2 mM), penicillin (100 units ml⁻¹) and streptomycin (100 µg ml⁻¹) under an atmosphere of 5% CO₂ in air. Cells were incubated with various concentrations of busulphan, quercetin or the two drugs in combination. Drugs were dissolved in DMSO and sterilised by filtration before addition to the tissue culture medium (the final concentration of DMSO was not more than 0.5%). Drugs were freshly made up for each experiment. After 5 days the cells were harvested and the viable cell number was determined by trypan blue exclusion. The ID₅₀, ID₇₀ and ID₉₀ values for quercetin alone and busulphan alone were 59, 106 and 265 µM and 13, 50 and 404 µM respectively, as determined from dose-response curves generated by the dose-effect analysis computer program (Figure 1).

The effects of mixtures of quercetin and busulphan on K562 cell proliferation were determined from median effect plots of different concentrations of the two drugs singly and combined in fixed ratios. These effects were quantitated in terms of the 'combination index' (CI) (Chou & Chou, 1987). CI=1 indicates summation, CI<1 indicates synergism, and CI>1 indicates antagonism. In order to compensate for variability between experiments, dose-response curves for

both individual drugs and mixtures were determined within a single experiment. Analysis of the data for quercetin and busulphan combined in a ratio of 1:1 indicated that the two drugs acted synergistically over a fractional effect range of 0.75-0.95 (75-95% cell kill) (Figure 2). The data have been analysed under mutually non-exclusive conditions since we have made the assumption that the two drugs are acting at different targets. Similar results were obtained when the data

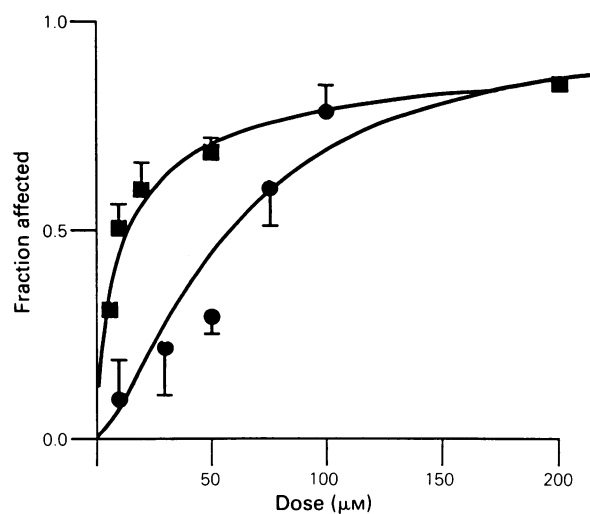


Figure 1 Dose-response curves of K562 cells treated with busulphan (■) or quercetin (●). Viable cell number was determined by trypan blue exclusion 5 days after treatment. Data represent the means (\pm s.d.) of three experiments.

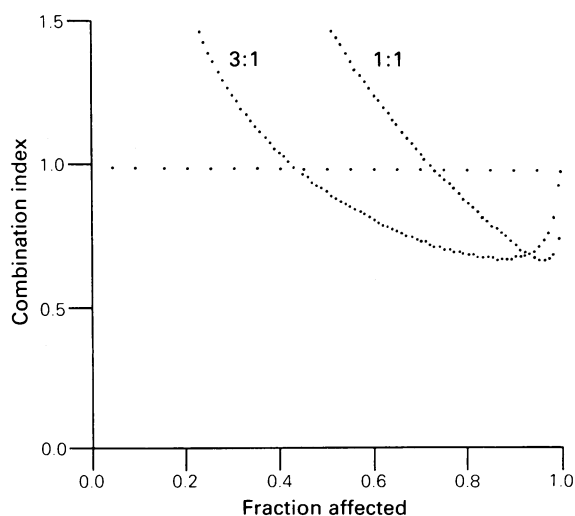


Figure 2 Effect of quercetin and busulphan combined 1:1 and 3:1 on K562 cell proliferation. Data were analysed under mutually non-exclusive conditions (see text). Data represent the means of three experiments.

were analysed under mutually exclusive conditions (i.e. the two drugs are assumed to act at the same site; data not shown). Increasing the ratio of quercetin:busulphan to 3:1 enhanced the synergism over the fractional effect range of 0.45–0.95 (Figure 2). The ID_{50} , ID_{70} and ID_{90} values for the 1:1 mixture and 3:1 mixture were 28, 60 and 204 μM , and 25, 53 and 182 μM respectively. Thus 18 μM quercetin combined with 6 μM busulphan was as effective as 13 μM busulphan in causing 50% inhibition of K562 proliferation.

Although the maximum tolerated dose for quercetin in humans has not been determined, plasma concentrations of

12 μM quercetin were achieved following an intravenous infusion of 100 mg without any apparent side effects (Gugler *et al.*, 1975). Interestingly, PKC inhibitors, of which quercetin is an example, may be effective against normally drug resistant cells (Wiesenthal *et al.*, 1987; Fine *et al.*, 1988). This property, together with the anti-proliferative effects of PKC inhibitors (O'Brian *et al.*, 1986), makes them attractive for development as anti-cancer agents either for use on their own or in combination with conventional cytotoxic drugs.

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