Short Communication

THE EFFECT OF MISONIDAZOLE COMBINED WITH WR2721 ON TUMOUR RESPONSE AND LEUCOPENIA DUE TO CYCLOPHOSPHAMIDE OR MELPHALAN

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There is increasing evidence that the cytotoxic action of certain chemotherapeutic drugs may be enhanced more in tumours than in normal tissues by combining them with misonidazole (MISO) (see reviews by Brown, 1982; McNally, 1982). This is particularly true of the alkylating agents and certain nitrosoareas. For instance, Martin et al., (1981) reported a dose-modifying factor (DMF) of 2 when 1 mg/g MISO was combined with cyclophosphamide (Cy) against the WHFIB tumour, with no effect of MISO on the leucopenia induced by Cy. A similar result was obtained with melphalan.

In contrast, the radioprotective drug WR2721 has been reported to protect certain normal tissues to a greater extent than tumours from the cytotoxic action of Cy (Yuhas et al., 1980; Twentyman, 1981), nitrogen mustard (Yuhas, 1979), cis-platinum (Yuhas & Culo, 1980), and a range of cytotoxic drugs including Cy, nitrogen mustard and cis-platinum (Wasserman et al., 1981).

I have therefore combined MISO and WR2721 with either Cy or melphalan in an attempt to increase the effectiveness of the chemotherapeutic drugs in tumours due to MISO potentiation, while reducing their effects in normal tissues due to WR2721 protection, thus increasing the therapeutic ratio. The normal tissue endpoint was white cell depression.

The tumour used was the WHFIB tumour growing in inbred WHT/GyfBSVS mice. This is a poorly differentiated sarcoma derived from a fibrosarcoma which arose spontaneously in a WHT mouse. It has been adapted for growth in vitro so that after treatment in vivo the response can be assessed in terms of tumour cell survival in vitro (George et al., 1977). The methods used to obtain tumours and to assay cell survival after treatment have been described before (Martin et al., 1981). Tumours were treated when they had reached a mean diameter of 6–8 mm, when the volume doubling time was about 3·5 days. The drugs were administered i.p. as a solution in normal saline, except for WR2721 which was dissolved in distilled water. They were dissolved immediately before use at a concentration such that the volume injected would be either 0·01 or 0·02 ml/g body wt.

Leucopenia induced by Cy or melphalan was assessed by drawing up a small volume of blood from the cut tip of the tail of a mouse lightly anaesthetized with penthrane into a heparinized capillary tube. The blood was then diluted in 0·2% acetic acid to lyse the red cells and the white cells were counted using a haemocytometer and phase contrast microscopy. At least 5 mice were used for each dose point.

It was shown previously that the cells of the WHFIB tumour are capable of recovering from the potentially lethal damage induced by Cy or melphalan (Martin et al., 1981). Consequently
The effect of Cy (MISO 500 mg/kg; 2721 400 mg/kg) (A) or melphalan (MISO 800 mg/kg; 2721 300 mg/kg) (B) on tumour cell survival of the WHFIB tumour. Symbols: Δ, chemotherapy drug alone; ×, drug + WR2721; ○, drug + MISO; ●, drug + MISO + WR2721.

TUMOUR RESPONSE TO MISO AND WR2721

Fig. 1.—The effect of Cy (MISO 500 mg/kg; 2721 400 mg/kg) (A) or melphalan (MISO 800 mg/kg; 2721 300 mg/kg) (B) on tumour cell survival of the WHFIB tumour. Symbols: Δ, chemotherapy drug alone; ×, drug + WR2721; ○, drug + MISO; ●, drug + MISO + WR2721.

MISOS and yet enough WR2721, on the basis of published results, to give chemoprotection if there is any. The doses of MISO and WR2721 were 500 and 400 mg/kg respectively with Cy and 800 and 300 mg/kg respectively with melphalan. The doses differed because whereas 500 mg/kg MISO potentiates the action of Cy upon the WHFIB tumour, a larger dose is needed with melphalan (McNally, unpublished data). MISO enhanced the action of Cy against the WHFIB tumour, giving a DMF of about 2 (Fig. 1(A)). WR2721 had no effect on Cy toxicity or its potentiation by MISO. With melphalan, MISO gave a DMF of about 2.4. Again there was no effect of WR2721 either with or without MISO.

The data points in Fig. 1 represent survival values for individual tumours (not all treated on the same day). No correction has been made for variations in cell yield because no systematic effect of the treatment on cell yield could be detected. MISO and WR2721 either alone
or in combination, at the doses used, had no effect on tumour cell survival.

The effect of Cy on the total white cell count in mice without tumours is shown in Fig. 2. Fig. 2(A) shows that the nadir in the white cell count was reached 3 days after injection of the Cy, with full recovery following a dose of 100 mg/kg by Day 7. Each data point in Fig. 2(A) represents the average white cell count for 5 mice. Previous studies have shown no effect of MISO on the extent of or the time course of the fall and subsequent recovery of the white cell count due to Cy (McNally et al., 1982). Neither MISO or WR2721 alone, nor the two together, had any effect on the nadir following 100 mg/kg Cy or on the subsequent recovery in the white cell count (Fig 2(A)). MISO and WR2721, either alone or in combination, also had no effect on control mice or the dose–effect curve for depression in white cell count at 3 days induced by Cy (Fig. 2(B)). The points in Fig. 2(B) represent the pooled results from 3 experiments each using 5 mice per point for Cy alone or with MISO, or 2 experiments when WR2721 was used. The error bars represent 2 standard deviations derived from the average white cell count for all mice pooled together. Neither MISO nor WR2721 either alone or combined had any effect on the white cell count of control mice.

Fig. 3 shows the effect of melphalan, either alone or with MISO or WR2721, or with both, on white cell depression assayed 5 days after injection. This had previously been found to be the time of the nadir in white cell count due to melphalan in this strain of mice (McNally et al., 1982). The points and error bars were obtained as described for Fig. 2(B). As with Cy, MISO or WR2721 separately had no effect on the white cell depression due to melphalan.

The combination of 800 mg/kg MISO and 300 mg/kg WR2721 was moderately
MISO and Cy or melphalan. The WR2721 protected neither the tumour nor the normal tissue from the toxic action of the chemotherapeutic drugs and had no effect on the potentiation of their toxicity by MISO in the tumour.

Yuhas (1980) and Yuhas et al. (1980) found no effect of 200 mg/kg WR2721 on the growth delay induced by Cy in the mammary carcinoma Mca-11, whereas it did protect the mice against the lethal effects of Cy increasing the LD$_{50/30}$ from 372 to 540 mg/kg. Twentyman (1981) found a minimal effect of 400 mg/kg on the LD$_{30/50}$ dose of Cy in mice although there was significant protection at 200 mg/kg. He also found minimal protection by 400 mg/kg on the leucopenia induced by 33 or 100 mg/kg Cy although there was some protection at 67 mg/kg. He also found some protection by WR2721 against the action of Cy upon the RIF-1 and KHT tumours (Twentyman, 1981). The overall conclusion was that there was minimal therapeutic gain from the use of WR2721 with Cy. In contrast, Wasserstein et al. (1981) measured a small protective effect of 600 mg/kg on the effect of Cy in EMT6 tumours, but a larger protective effect on the bone marrow cells, leading to a significant therapeutic gain.

The present results suggest that there is no differential protection against Cy or melphalan by WR2721 when comparing effects on the WHF1B tumour and leucopenia in WHT mice. Also WR2721 did not affect the potentiation of the action of these drugs by MISO against the WHF1B tumour. There is evidence that at a lower dose (200 mg/kg) WR2721 may show a greater protective effect in normal tissues (Twentyman, 1981), although in the present study it had no protective effect at doses at 300 and 400 mg/kg.

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