COMPARATIVE EFFECTS OF CYCLOPHOSPHAMIDE AND ISOPHOSPHAMIDE ON LEWIS LUNG CARCINOMA

A. CORSI, F. CALABRESI AND C. GRECO

From the Regina Elena Institute for Cancer Research, Rome, Italy

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ISOPHOSPHAMIDE (IP) is a new antineoplastic drug, chemically related to cyclophosphamide (CP) by transposition of a chloroethyl group from the exocyclic to endocyclic nitrogen. Recently, extensive clinical investigations (Cohen & Mittleman 1973, Creaven et al., 1976) have praised the lower toxicity of IP than of CP. Furthermore, previous experimental data are very encouraging. They demonstrate that the antitumour effect of IP is similar to, or higher than, that of CP when assayed on some experimental tumours of the rat, such as DS-carcinosarcoma (Von Ardenne et al., 1971) Yoshida sarcoma (Brock, 1972) and Jensen sarcoma (Van Dyk et al., 1972).

Therefore, in order to verify this difference and to estimate the relative efficacy of the 2 alkylating agents, we have compared the toxicity (expressed as LD$_{50}$) and the therapeutic effectiveness of CP and IP on Lewis lung carcinoma (3LL) after a single dose 5 days after tumour implantation.

Our experimental model was implanted i.m. in C57BL mice with $5 \times 10^5$ viable tumour cells. The tumour was aseptically removed from the leg and made into a cell suspension as previously described (DeWys, 1972). Tumour growth was calculated by caliper measurement of diameters, and the Wexler procedure was used for metastasis counting (Wexler, 1966). CP and IP, dissolved in double-distilled water, were injected i.p. in a volume of 0.01 ml/g body weight.

The toxicity expressed as LD$_{50}$ has been 360 mg/kg for CP and 540 mg/kg for IP, which agrees with the literature.

The following quantitative endpoints were used to assess antitumour effectiveness of the drugs:

(i) tumour growth inhibition (T/C) obtained by caliper measurements as described above, and

(ii) tumour growth delay in days (T–C) to a predetermined size (1g).

As shown in the Table, both CP and IP revealed a dose-dependent chemotherapeutic activity when administered in single doses to 3LL-bearing mice.

In particular, after CP and IP given i.p., the i.m. implanted 3LL exhibits a very remarkable inhibition of the primary tumour growth at increasing doses up to a “cure” after 300 mg/kg CP and 500 mg/kg IP.

Response of 3LL to CP and IP was also evaluated by the time required for the treated tumours to reach the size of 1g.

Data reported in the Table show the growth delay of the tumours after chemotherapy relative to tumours in the control group. As can be seen, although tumour-growth retardation was shown by tumour-bearing mice after both CP or IP (at all concentrations tested) a greater effect of CP than IP at the same active dose was found. In particular, after 300 mg/kg CP, tumour growth was arrested, and mice showed a complete disease-free survival over 90 days, being considered “cured”.
An equal dose of IP arrested the tumour growth for a remarkably long period (17 days) but it was not "curative". A complete disease-free survival was obtained only after a 500 mg/kg IP treatment.

In further experiments, the effect of CP and IP on lung metastases was investigated. As illustrated in the Table, single doses of CP and IP 5 days after tumour implantation induced a significant reduction in the number of lung nodules. Again, a total disappearance of metastases was found only after a dose of IP 1.6 times that of CP.

The purpose of this research was to verify preliminarily the therapeutic and toxic effects of IP vs its analogue, CP on 3LL tumour. This experimental model has been chosen both because of its well-known response to CP and of the similarity of its particular metastasizing behaviour to that of human tumours.

Preliminary clinical investigations seemed to demonstrate that IP exhibited a less toxic effect than CP, maintaining, on the other hand, a similar therapeutic effectiveness.

Our preliminary results reported here do not support a real advantage of IP over CP in their respective balances between toxic and therapeutic effects. In fact, LD<sub>50</sub> IP/LD<sub>50</sub> CP = 1.5 but the dose of IP necessary to reach the same "curative" effect on 3LL tumour is 1.6 times that of CP.

Because of the clinical interest of these experimental data, we are performing further studies on 3LL, using different schedules, including those for adjuvant therapy after surgery, in order to draw more general conclusions on the claimed advantage of this derivative of CP.

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**TABLE. — Effect of single cyclophosphamide and isophosphamide doses on Lewis lung carcinoma**

| Treatment | Drug | Dose (mg/kg) | Average wt.† (g ± s.e.) | T/C‡ (days) | T−C | Effect on Metastases | % ILS | Tumour-free survivors at 90 days (%) |
|-----------|------|-------------|--------------------------|-------------|-----|---------------------|-------|-----------------------------------|
| Control   |      | 5 ± 23 ± 0.72 |             |             |     |                     |       |                                   |
| CP        |      | 100         | 4.11 ± 0.71             | 0.78        | 4   | 0.84               | 22.5  | 0                                 |
|           |      | 200         | 0.50 ± 0.21             | 0.09        | 16  | 0.62               | 68.5  | 33                                |
|           |      | 300         | $§$                      | $§$         | $§$ | 0                  | >261.2|                                   |
| IP        |      | 100         | 5.83 ± 0.70             | 1.11        | 0.6 | 0.64               | 10.8  | 0                                 |
|           |      | 200         | 2.31 ± 0.47             | 0.44        | 0.95| 0.65               | 29.8  | 0                                 |
|           |      | 300         | 0.46 ± 0.40             | 0.08        | 17  | 0.66               | 110   | 20                                |
|           |      | 400         | 0                       | 0           | 25  | 0.52               | 128   | 60                                |
|           |      | 500         | $§$                      | $§$         | $§$ | 0                  | >261.2|                                   |

* Single doses were given 5 days after i.m. transplant of 5 × 10⁵ viable tumour cells.
† Tumour weights at the median day of control death (25 days).
‡ Ratio between the average values of each treated group and the relevant control. $§$ Treated tumours remain below the limit of palpation for 90 days.
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