Cancer Chemother Pharmacol (1983) 11:200–202

Cancer Chemotherapy and Pharmacology

Short communication

The testis

A protected environment for leukaemic cells against cyclophosphamide in a mouse model

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Summary. *Groups of BDF_1 mice, inoculated either IM or by the intratesticular (IT) route with comparable numbers of L1210 cells, died within the same time range from the disseminated disease. Cyclophosphamide (100 mg/kg IP) given on day 6 after inoculation, when the disease was advanced, increased the lifespan by about 100%, but all the mice died. The same dose on day 3 effectively cured all mice inoculated IM, whereas those injected with cells into the testicular lymphatic sinusoidal system died with only a short prolongation of lifespan. The study indicates that L1210 cells present in the testis are relatively protected from the action of cyclophosphamide, and the experimental results are consistent with clinical evidence for the occurrence of relapse in children with ALL due to malignant lymphoblasts persisting in the testicular environment.*

Clinical experience over a number of years has led to the belief that the testis provides an environment in which malignant lymphoblasts can remain relatively protected from chemotherapeutic attack. With the effective combating by irradiation of the meningeal manifestations of acute lymphoblastic leukaemia, the male gonad has become increasingly suspect as a site of relapse following chemotherapeutically induced remission. The use of cyclophosphamide is considered possibly even to favour the incidence of testicular disease [4]. In extensive neoplastic disease the cells can enter sanctuaries in which drug concentrations never reach levels adequate to eradicate the tumour. In addition to known sanctuaries within the central nervous system other areas, such as the thymus and the gonads and the poorly perfused interior of large solid tumours, may be important [8].

Our experiments with a mouse model system have now provided convincing evidence that leukaemic cells within the male gonadal environment are rendered less vulnerable to elimination by systemic chemotherapy with cyclophosphamide. Using the well-known L1210 leukaemia transmitted by IM inoculation in the BDF_1 mouse strain, we have failed over many months and under a variety of experimental circumstances to obtain histological evidence that these circulating leukaemic cells are capable of penetrating the walls of the testicular blood vessels and so gaining entry to the intertubular system. However, when micro-doses of leukaemic cells (about 5,000 cells in 2 µl) are injected intratesticularly (IT) into the sinusoidal lymphatic system, they proliferative rapidly, disseminate, and cause death in about the same time range (9–13 days) as a comparable dose of cells given IM in a hind limb. The histological appearance of the inoculated testis towards the terminal phase closely resembles that seen in children with acute lymphoblastic leukaemia.

In a series of experiments, comparable groups of mice each inoculated by one of these two routes and given a single IP dose of cyclophosphamide (100 mg/kg) on the third day afterwards showed a remarkable difference in response. As mentioned above, the untreated controls all died within similar periods of time irrespective of the mode of inoculation. However, the IT-inoculated, cyclophosphamide-treated groups lived a few days longer, whereas the IM-inoculated mice similarly treated with the drug all survived for many months and could be regarded as cured (Table 1). That the leukaemic cells had been eliminated in these latter animals was further emphasised by the fact that cell suspensions from their spleens failed to induce leukaemia when injected into groups of BDF_1 mice. Not only are L1210 cells 100% lethal in routine weekly transmissions of the disease using splenic suspensions, but it has been demonstrated that very few cells are required consistently to reproduce the disease [9].

The response of the L1210 leukaemia to a single dose of cyclophosphamide nevertheless depends essentially on the number of cells injected and the subsequent incubation time before the drug is administered. Thus, in the present series, leukaemic mice treated on day 6 all died although there was an obvious increase in survival time (Table 2), which led to the choice of a 3-day post-inoculation time (Table 1). It is interesting that with BDF_1 mice and SC inoculum of 10^7 L1210 cells it was found that even with 245 mg/kg of cyclophosphamide SC on day 3 the proportion of 60-day survivors only approached 80% [5], in contrast to the 100% cures in the present experiments with 100 mg/kg (Table 2). The only other difference between the results published [5] and the present experiments lies in the route of administration of the drug, SC vs IP, respectively. It is possible that the intra-abdominal route of injection leads to a higher proportion of biotransformation of cyclophosphamide in the liver to its active metabolite and hence a more effective therapeutic response. The size of the initial inoculum of leukaemic cells, in terms of cell numbers, in our experiments had little influence on the fatal outcome, e.g., 8–11 days with 2 µl of splenic suspension, compared with 8–9 days with 50–100 µl (5×10^5 cells) in routine passage of the leukaemia.

The interpretation of the present work must surely be that the leukaemic cells introduced into the mouse testicular interstitium are relatively protected in some way from the action of this cytotoxic drug. The testicular capillary endo-

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Table 1. Survival of leukaemic mice inoculated IM or by intratesticular (IT) route[a]

No. of mice	Route of inoculation	Treatment	Survivors	Range of deaths (days)	Mean survival time (days)
Experiment 1					
8	IM	None	0	10–11	10.5
7	IM	Cyclophosphamide	7	–	> 8 months
8	IT	None	0	9–11	10
7	IT	Cyclophosphamide	1[b]	15–17	16
Experiment 2					
8	IM	None	0	10–12	10.5
7	IM	Cyclophosphamide	7	–	> 8 months
8	IT	None	0	9–11	10
7	IT	Cyclophosphamide	1[b]	15–17	16
Experiment 3					
11	IT	Cyclophosphamide	2[b]	17	17

[a] Male BDF_1 mice were inoculated with 2 µl (approx. 5,000 cells) of a splenic suspension from a leukaemic male; cyclophosphamide 100 mg/kg IP was given on day 3

[b] Probably failed IT injections

Table 2. Survival of leukaemic mice inoculated by IM or IT injection and treated on day 6[a]

No. of mice	Route of inoculation	Treatment	Survivors	Range of deaths (days)	Mean survival time (days)
8	IM	None	0	8– 9	8.5
7	IM	Cyclophosphamide	0	17–24	20
8	IT	None	0	9–10	9.5
7	IT	Cyclophosphamide	0	16–22	17

[a] When treatment was delayed from day 3 to day 6 there were no survivors regardless of the route of inoculation of L1210 cells, although the lifespan was about twice that of control animals

[b] Cyclophosphamide 100 mg/kg

thelium is known to be readily permeable to various macromolecules and drugs, including cyclophosphamide. There is no reason to suppose that this compound, or its active metabolite, is hindered from access to the testicular system. On day 3 after the testicular inoculation, when the drug was administered IP, leukaemic cells could not be positively identified histologically in the mouse testis, so there was no question of a physical block by tumour cells to drug access. By day 6 there was ample evidence of peritubular proliferation by leukaemic cells in untreated mice, although the seminiferous epithelium appeared intact.

This experimental investigation supports the clinical view that the testis can provide a so-called pharmacological sanctuary for leukaemic cells, with particular reference to cyclophosphamide. Various suggestions have been put forward to explain the clinical phenomenon of testicular relapse. These include variations in the drug combinations used during treatment, possible antagonism between drugs, and actual stimulation of neoplastic proliferation. For cyclophosphamide there is a specific speculation that its ability to destroy spermatogenic cells within the seminiferous tubules (i.e., beyond the blood-testis barrier) may change the micro-environment to allow proliferation of leukaemic cells in that site [4, 6]. In connection with the latter it is our experience with lymphoblastic cells actively proliferating in the mouse and rat intertubular environment that the seminiferous tubular barrier is not penetrated by leukaemic cells. Rather, the peritubular infiltration eventually causes tubular atrophy. A toxic drug action might alter the situation and this is being investigated. The possibility has also been raised that leukaemic cells may persist preferentially in the testis because of local immunological factors [3], and that tissue damage after chemotherapy, e.g., testicular interstitial fibrosis [7], might enable leukaemic cells to survive in a milieu in which the drug concentration will be reduced [3]. In the current mouse experiments the latter suggestions provide no explanation. Another factor is the well-known low testicular-epididymal temperature compared with the body [1]. The reactivity of cytotoxic chemicals is temperature-dependent and this could contribute to a 'sanctuary' effect, in much the same way as scalp hair loss has been prevented by cooling during daunomycin treatment [2]. It is evident that in the present experimental ALL system an appropriate balance between the factors of cell population, time, and drug-exposure dose are necessary to demonstrate the existence of a relatively protected environment within the male reproductive system.

The incidence of testicular relapse in children with ALL could likewise depend upon the interplay of a number of factors – the malignant lymphoblast population when inductive treatment commences, the cell type of the leukaemia, the combination of drugs used (involving possibilities of synergism and antagonism), the individual metabolic capacity of the patients to detoxicate or activate drugs, and the testicular temperature differential.

Acknowledgements. This work forms part of a general experimental investigation of testicular relapse in acute lymphoblastic leukaemia and possible chemotherapy initially undertaken at the suggestion of Dr P. Morris Jones. The financial support of the Leukaemia Research Fund is gratefully acknowledged.

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Received June 30, 1983/Accepted August 3, 1983