CYCLOSPORINE - A MODEL FOR THE TESTING OF BIO-IMMUNOLOGICAL REACTION MODIFIERS IN MAN

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Cyclosporine is a member of a new family of drugs which interfere and modify the biological regulation of the immune system without being cytotoxic and has now been tested extensively in man. Observation of its toxicity in a homogeneous group of 72 patients with acute myeloblastic leukaemia receiving bone marrow transplants serves to illustrate some of the new and unexpected problems that may be seen when BIRM agents start to be tested in the clinic.

Key words: Cyclosporine, BIRM, Man.

INTRODUCTION

Although Cyclosporine is not in a strict sense a bio-immunological reaction modifier (BIRM) it is the first immunological agent to become available that is not cytotoxic against lymphocytes. There is some contention at present about whether the action of Cyclosporine is due to a specific effect on helper T-lymphocytes or alternatively due to blocking of the transmission of antigen mediated signals within the cell. Nevertheless, it has already been established in the clinic as a profound immunosuppressive agent. The purpose of this present paper is to describe the information obtained in the use of this drug in our Unit over a period of 6 yr from the time when the first patient was treated in a Phase 1 study to the licensing of the agent and to see what lessons can be learnt that may be applied to the testing of BIRM agents that are now available for Phase 1 studies.

PATIENTS AND METHODS

Seventy-two patients with AML received BMT during first remission at the Royal Marsden Hospital between August 1978 and May 1983. The age range was 4-46 yr with a median of 37 yr.

Conditioning regimen

Fifty-six patients received cyclophosphamide

60 mg/kg i.v. daily for 2 days followed after 36 h by total body irradiation (TBI) of 10 Gy given as a single dose at a dose rate of approximately 1.5 Gy h⁻¹. An additional 11 patients were conditioned with a priming intravenous dose (i.v.) of cyclophosphamide 300 mg m⁻² followed 7 days later by a single i.v. dose of Melphalan (85-100 mg m⁻²) and 12 h later TBI at a dose of 10 Gy. Both these groups of patients received the marrow transplant the day after the TBI. A third group of five patients received the same priming dose of cyclophosphamide, followed 7 days later by a single dose of Melphalan 240 mg m⁻² (and no TBI), and 4 days after the Melphalan they received their transplant.

Bone marrow transplant

Approximately 2.0-3.5 × 10⁸ kg⁻¹ nucleated bone marrow cells were given from HLA/DR matched sibling donors. The marrow was not subjected to any separation procedures prior to infusion.

Cyclosporine (CYA)

Five different regimens were used. Between August 1978 and September 1982, 38 patients received intramuscular CYA 25 mg kg⁻¹, from the day prior to transplant for 5 days followed by oral CYA 12.5 mg kg⁻¹ for 175 days. During the same period an additional 3 groups of patients received oral instead of i.m. regimens as follows; from the day prior to transplant CYA was given for 5 days at a daily dose of either 8 mg kg⁻¹ (7 patients),

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12.5 mg kg\(^{-1}\) (15 patients), or 35.5 mg kg\(^{-1}\) (8 patients) followed by a maintenance dose of 8–12.5 mg kg\(^{-1}\) for 175 days. In all studies the maintenance dose of CYA was titrated against toxicity with the dose being reduced by 33% if the creatinine rose about 250 \(\mu\)mol l\(^{-1}\) or the BUN was above 20 mmol l\(^{-1}\). For approximately 70% of the patients the radioimmunoassay of CYA was available and serum levels were monitored and kept within a 'therapeutic' range of 500–1000 ng ml\(^{-1}\). Since September 1982 when i.m. CYA was no longer available four patients have received 12.5–25 mg kg\(^{-1}\) day\(^{-1}\) infused over 12 h for the first five days of transplant.

Forty of the 72 patients in the series have stopped taking CYA at 180 days post transplant.

**Graft versus host disease**

Initially all patients developing skin rashes had a biopsy taken, but no change was made in management and in all but one instance there was complete resolution of the skin problem. After our first fatality with GVHD all patients with skin GVHD received as treatment three days of i.v. Methylprednisolone 700 mg m\(^{-2}\). Recently skin biopsies have no longer been performed.

**RESULTS**

**Graft versus host disease**

Of the 38 patients receiving i.m. CYA 26 (68%) are alive; 71% either had proven or suspected GVHD and one (3%) died of the syndrome. There was no significant difference in the results obtained for the 30 patients treated only with oral CYA; 56% are alive, 66% developed suspected or proven GVHD and one (3%) died from it.

**Survival**

Forty-seven of the 72 patients remain alive with a projected actuarial plateau between 26 and 56 months at 54%. Of the 25 deaths, 8 (11%) were due to recurrent leukaemia (day 92–393; median 189), 6 due to pulmonary oedema (day 30–513; median 89), four due to renal failure (day 27–84), 3 pneumonia (day 41–65), 2 GVHD (day 49, day 77) and 1 CNS (day 20) and 1 carcinoma of the rectum (day 698). There were thus 23% transplant related deaths. Four of the 8 patients receiving high initial CYA doses (35.5 mg kg\(^{-1}\) day\(^{-1}\)) died of pulmonary oedema whereas there were only 2 deaths from this cause in the whole of the rest of the series. Otherwise the causes of death could not be related to the CYA administration. Although renal dysfunction occurred in over 70% of patients on CYA, death due to renal failure was not directly attributable to CYA (or serum levels). However, a correlation was found between the condition-

ing regimen and severe renal failure; only 1 patient died of renal failure of 56 conditioned with cyclophosphamide and TBI, whereas 3 of 16 patients who received Melphalan (with or without TBI) died of the problem.

Age was the most important factor associated with survival; of 23 patients under 20 yr of age 78% remain alive; of 18 patients between 21 and 30 yr 67% are alive; of 24 patients between 31 and 40 yr 59% are alive and only 43% are alive of seven patients over 40 yr of age.

The serum bilirubin was also of prognostic significance; 59 patients had bilirubin levels remaining less than 100 \(\mu\)mol l\(^{-1}\) (normal <17 \(\mu\)mol l\(^{-1}\)) during the first 12 weeks of transplant and 43 (73%) are alive whereas only 3 (24%) of 13 patients are alive whose bilirubin rose above 100 \(\mu\)mol l\(^{-1}\). The cause of death of the 10 severely jaundiced (conjugated) patients was not liver failure; 3 died of pulmonary oedema, 3 of infection, 2 of renal failure, 1 of GVHD and 1 of carcinoma of the rectum.

**Stopping CYA**

Forty patients stopped taking CYA after 180 days and 12 required reinstition of the drug within 60 days due to the development of acute GVHD in either skin or liver (based on liver function tests). All had resolution of their GVHD.

**DISCUSSION**

It was gratifying in this present series to find that only 3% of patients died as a direct consequence of graft versus host disease and gave us the opportunity in a homogeneous group of patients of seeing the sort of problems that may unexpectedly occur when non-cytotoxic agents that interfere with the biological regulation of the immune system are used for prolonged periods of time. This is particularly important because 'non-immunological' functions of lymphocytes are now being described (including the maintenance of the integrity of the vascular endothelium) which may lead to serious toxic side effects of BIRM agents and was a source of concern in this present series of transplant patients where there was still a relatively high proportion (23%) of deaths due to causes other than leukaemia. Fatal pulmonary oedema (a problem which dominated our mismatch transplant programme\(^{4}\)) was the most common serious occurrence and seems almost certainly due to increased vascular permeability related to the administration of CYA although no direct correlation could be found between the dose of CYA, the serum levels of CYA and this complication.\(^{6}\) Increased vascular permeability probably also occurs in the brain in patients on CYA and several of the younger patients had convulsions during the first 4 weeks after transplant. It is of major concern that drugs