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COMBINATION CHEMOTHERAPY WITH CIS-DIAMMINE-DICHLORO-PLATINUM, VINBLASTINE, AND BLEOMYCIN IN ADVANCED TESTICULAR NON-SEMINOMA

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Summary 40 patients with disseminated testicular non-seminoma were treated with *cis*-diammine-dichloro-platinum, vinblastine, and bleomycin. Complete remission was achieved in 24 (60%) patients and partial remission in 11 (28%). 22 of the 24 complete responders, who have been followed-up for a median of 11 months, have been tumour-free for 5–30 months. There were 3 drug-related deaths. This regimen is the most effective remission-induction treatment available for disseminated testicular non-seminoma. Patients should be treated in centres experienced in the specialised management of this potentially curable disease.

Introduction

TESTICULAR neoplasms are rare, their annual incidence ranging from 0·9 to 4·5 per 100 000 males in Europe, and they occur most commonly in the 20–40 year age-group. They are the main cause of cancer death in men aged 25–34 years.[1,2]

Seminomas and non-seminomas comprise 85–95% of all testicular tumours.[2,3] When seminomas are treated by radiotherapy to the regional lymph-nodes after orchiectomy the cure-rate is 90–95%.[4,5]

Until recently, the prognosis of disseminated non-seminoma was very poor. Combination chemotherapy with actinomycin-D, methotrexate, and chlorambucil resulted in 10–20% complete remissions.[6–9] These results were improved when Samuels introduced vinblastine (V.L.B.) and bleomycin (B.L.M.) and obtained complete remissions in 39% and partial remissions in 35% of a series of consecutive cases.[10–12] The encouraging results obtained with *cis*-diammine-dichloro-platinum (C.D.D.P.)[13–18] in phase I[19,20] and phase II[21,22] studies in testicular non-seminoma led to its use in combination chemotherapy. In 1976 Einhorn reported the first results of C.D.D.P., V.L.B., and B.L.M. remission-induction therapy,[23] followed by maintenance on V.L.B. and B.C.G. immunotherapy, The promising results were subsequently confirmed, and in 1978 he reported 33 (70%) complete and 14 (30%) partial remissions in 47 patients. 27 patients were still in complete remission after a follow-up of 26–49 months.[24–26] Better remission-rates were not obtained by adding other drugs to this combination.[27–30]

In June, 1976, we started a study of C.D.D.P., V.L.B., and B.L.M. in patients with stage III non-seminoma. The maintenance therapy consists of C.D.D.P. and V.L.B., scheduled for 2 years.

Patients and Methods

40 patients, aged 17–53 years (mean 32 and median 29), with disseminated testicular non-seminoma have been given remission-induction chemotherapy consisting of C.D.D.P., V.L.B., and B.L.M.

All patients had measurable lesions except 1 in whom a raised serum-level of the β subunit of human chorionic gonadotrophin (β-H.C.G.) was the sole indicator. The patients were classified according to Pugh[2] (table I). The extent of spread of metastases was assessed according to Samuels.[11] Table II shows that most of the patients had advanced abdominal and/or advanced pulmonary disease. 50% of them had previously been treated with radiation and/or chemotherapy for metastases; these included 2 who had received V.L.B. and B.L.M. and 1 who had received V.L.B. and B.L.M. followed by C.D.D.P., 'Adriamycin' (doxorubicin), and cyclophosphamide.

After prehydration with 1 litre of saline, C.D.D.P., 20 mg/m^2 in 300 ml 15% mannitol, was infused over 2 h for 5 consecutive days. Three to four cycles were given, the next cycle starting on day 22 of the previous cycle. During each treatment cycle, diuresis was maintained by at least 4 litres of saline/24 h. V.L.B. was given as an intravenous bolus on days 1 and 2 of each treatment cycle in a dose of 0·2 mg/kg daily. B.L.M. 30 U intravenously was given in a 15 min infusion 6 h after the V.L.B. on day 2 of each cycle and also at weekly intervals between cycles until a total dose of 360 U had been given. The sequential use of V.L.B. and B.L.M. is based on experimental data obtained in cell-cycle kinetic studies and animal models.[31,32]

After remission-induction chemotherapy complete responders were maintained on intravenous V.L.B. (0·3 mg/kg), alternating with intravenous V.L.B. (0·2 mg/kg) plus intravenous C.D.D.P. (50 mg/m^2) at 3 week intervals for 2 years. Patients were admitted for 1 day every 6 weeks for the C.D.D.P.-V.L.B. combination. The C.D.D.P. was provided by the

National Cancer Institute, National Institutes of Health, Bethesda, Maryland, U.S.A.

Complete remission was defined as complete disappearance for at least 8 weeks of all clinical, radiographic, and biochemical evidence of disease, which included the results of whole-lung tomography, computerised tomographic scanning of the abdomen, exploratory surgery if indicated, and assays of β-H.C.G. and α-fetoprotein (A.F.P.). Partial remission was defined as a decrease of 50% or more in the sum of the products of the perpendicular diameters of all measurable lesions for at least 8 weeks. If the disease progressed under this regimen, the case was classified as one of "progression" and the patient was excluded from the study.

Results

24 (60%) of the 40 patients achieved complete remission, 11 (28%) patients achieved partial remission, and 3 patients died of toxicity (table I). 2 of the 3 deaths occurred during remission-induction treatment; 1 was due to agranulocytic sepsis and the other to myocardial infarction (see below). The third patient died of B.L.M. lung fibrosis 6 months after completion of remission-induction chemotherapy, when he was still in complete remission. The lung fibrosis had developed when he was still on B.L.M. 2 other patients, who showed enlargement of lung metastases during remission-induction treatment, were classified as cases of progression and dropped from the study.

22 of the 24 complete responders, who have been followed-up for an average of 13 months (median 11 months), have remained disease-free for 5–30 months. 1 of the other 2 patients showed recurrence of the disease after 7 months of complete remission: the recurrence was accompanied by a rise of lactic dehydrogenase (L.D.H.) and β-H.C.G. levels. At laparotomy, retroperitoneal lymph-node metastases were found. After irradiation of these metastases, the level of tumour markers became normal. The other patient was operated on, after 2 cycles of remission-induction chemotherapy, to reduce the size of a large differentiated teratoma in the abdomen, which recurred after 6 months of complete remission.

7 of the 11 partial responders showed progression of the disease after 3–5 months. 4 patients are still in partial remission after a follow-up of 6–15 months. Poor prognosis seems related primarily to the extent of the disease (table II). The preponderance of patients with advanced pulmonary and advanced abdominal disease is striking; those with advanced abdominal disease have the worst prognosis.

None of the histological types showed a preponderance of advanced disease (table III). In 30 (75%) patients tumour markers such as β-H.C.G. and/or A.F.P. were present. The presence of tumour markers was commoner in patients with advanced abdominal disease than in patients with advanced pulmonary disease (table IV).

Patients who had previously received radiotherapy were at greater risk of severe and prolonged hæmatological toxicity (table V). Septic shock and the treatment of sepsis with gentamicin and cephalothin contributed to the development of renal failure, which is a major side-effect of C.D.D.P. Of the 20 patients who had been treated previously, only 9 (45%) achieved a complete remission, compared with 16 (80%) out of 20 in the non-pretreated patients. The poorer prognosis in patients with radiotherapy was partially due to an increase in the number and severity of side-effects of chemotherapy, which can be reduced by decreasing the V.L.B. dose.

TABLE I—RESULTS OF CHEMOTHERAPY IN RELATION TO HISTOLOGY

Histological* diagnosis	No. of patients	Outcome†			
		C.R.	P.R.	T.D.	Progression
M.T.I.	9	6 (67%)	3	0	0
M.T.U.	24	15 (63%)	5	3	1
M.T.T.	7	3 (42%)	3	0	1
Total	40	24 (60%)	11 (28%)	3	2

*M.T.I.=malignant teratoma, intermediate; M.T.U.=malignant teratoma, undifferentiated; M.T.T.=malignant teratoma, trophoblastic.
†C.R.=complete remission; P.R.=partial remission; T.D.=toxic death.

TABLE II—RESULTS OF CHEMOTHERAPY IN RELATION TO EXTENT OF DISEASE

Extent of disease	No. of patients	Outcome*			
		C.R.	P.R.	T.D.	Progression
Minimum pulmonary disease	2	2	0	0	0
Advanced pulmonary disease	16	11	3	2	0
Minimum abd.+pulm. disease	1	1	0	0	0
Advanced abdominal disease	20	9	8	1	2
β-H.C.G. as sole parameter	1	1	0	0	0

*C.R.=complete remission; P.R.=partial remission; T.D.=toxic death.

TABLE III—RELATION BETWEEN HISTOLOGICAL DIAGNOSIS AND EXTENT OF DISEASE AT PRESENTATION FOR C.D.D.P. COMBINATION CHEMOTHERAPY

Extent of disease	No. patients with:		
	M.T.I.	M.T.U.	M.T.T.
Minimum pulm. disease	1	1	0
Advanced pulm. disease	3 (33%)	10 (42%)	3 (43%
Minimum abd.+pulm. disease	0	0	1
Advanced abd. disease	5 (56%)	12 (50%)	3 (43%
β-H.C.G. as sole indicator	0	1	0
Total no. of patients	9	24	7

TABLE IV—RELATION BETWEEN EXTENT OF DISEASE AND TUMOUR-MARKER STATUS

Extent of disease	No. of patients	Tumour markers*	
		Positive	Negative
	30	10	
Minimum pulm. disease	2	2	0
Advanced pulm. disease	16	8	8
Minimal abd.+pulm. disease	1	1	0
Advanced abd. disease	20	18	2
β-H.C.G. as sole indicator	1	1	0

* Markers are considered positive when β-H.C.G. is >4 ng/ml and/or A.F.P. is >16 ng/ml.

TABLE V—SIDE-EFFECTS OF CHEMOTHERAPY IN RELATION TO PREVIOUS THERAPY

Side-effects	No. of patients	Pre-treatment* R.T.± chemo (n=12)	Chemo only (n=8)	None (n=20)
Granulocytopenia†	15	10	2	3
Thrombocytopenia‡	9	9	0	0
Sepsis	11	8	2	1
Renal failure§	13	7	3	3

*R.T.=radiotherapy; chemo.=chemotherapy.
†<500×10⁶ per litre for >5 days.
‡<50 000×10⁶ per litre for >5 days.
§Serum-creatinine >120 mmol/l.

19 patients were given four cycles of C.D.D.P., V.L.B., and B.L.M. In 12 complete responders the fourth cycle was intended as consolidation therapy, required because of the large size of the original tumour and/or excessively raised level of tumour markers present at the beginning of chemotherapy. The other 7 patients, who had only partial remission after three cycles, did not achieve complete remission with a fourth cycle.

Exploratory surgery was done in 17 patients during or after the completion of the chemotherapy to reduce tumour bulk (1 patient) and to assess response to treatment. Investigation of previously affected lymph-nodes by laparotomy was done in 13 patients and revealed fibrosis (5 patients), necrosis (2 patients), normal architecture (3 patients), benign differentiation of the tumour (1 patient), and a resectable polycystic necrotic mass with a diameter of 10 cm (1 patient). This last patient also had a large supraclavicular lymph-node swelling which showed fluctuation after two cycles of chemotherapy, and which at dissection appeared to be a polycystic necrotic tumour, 8 cm in diameter. However, histological examination showed intermediate malignant teratoma cells in the peripheral layer of both the abdominal and the supraclavicular masses. Neither of these tumours shrank during chemotherapy. The 13th patient was rendered disease-free by resection of a small residual retroperitoneal lymph-node metastasis after four cycles of chemotherapy (he is classified as a partial responder).

Exploratory thoracotomy was done in 3 patients after chemotherapy because of a solitary residual metastasis in 2 and a more than 50% reduction of pulmonary metastases in the third. The pulmonary metastases in this last patient consisted of differentiated and intermediate malignant teratoma. In 1 of the other 2 patients the lesion was a hamartoma; no residual tumour was demonstrated in the second. Dissection of a residual supraclavicular tumour disclosed fibrosis in the 17th patient.

Both of the patients previously treated with V.L.B.-B.L.M are now in complete remission, which has lasted 12 months. The patient pretreated with C.D.D.P.-cyclophosphamide-'Adriamycin' has had a partial remission for 3 months with impressive relief of severe pain.

TOXICITY

The side-effects of C.D.D.P. combination therapy have been described in detail.[33–38] We present case-reports of 3 patients who died from complications of the combined C.D.D.P., V.L.B., and B.L.M. therapy.

Case 1

A 34-year-old man had a left-sided orchiectomy (M.T.U. plus seminoma) and radiotherapy (4000 rad) to the regional lymph-nodes in 1976, followed by further radiotherapy 2 months later for a lung metastasis. 4 months later he was started on chemotherapy for a right hilar mass. Side-effects during the first cycle included vomiting, leucopenia, thrombocytopenia, stomatitis, and B.L.M.-induced fever.

With the second treatment cycle he had paralytic ileus, agranulocytosis, septic fever (although repeated blood-cultures were negative), and severe stomatitis *(Candida albicans)*. He was treated with gentamicin and cephalothin and topical nystatin. Anuria developed; he was hæmodialysed, and renal function recovered after 2 weeks. Meanwhile, severe diarrhœa developed and *Candida albicans* was cultured from the stools; he was then treated with intravenous miconazole.

His persistent fever, agranulocytosis, and anæmia gradually improved with leucocyte and red-cell transfusions; his hearing loss, which occurred after treatment with gentamicin and cephalothin, disappeared; and the right hilar lung metastasis regressed.

The third and fourth treatment cycles had been delayed and was given in reduced doses. 6 months after the achievement of complete remission, he had dyspnœa, probably due to B.L.M. pneumonitis. B.L.M. was immediately withdrawn (by then the patient had received a total of 240 U). Prednisone did not relieve symptoms. Subcutaneous emphysema and a pneumomediastinum developed, the lung infiltrates spread, and he died a week later of progressive respiratory insufficiency. Necropsy was refused.

Case 2

A 32-year-old man had a left-sided orchiectomy (M.T.U. plus M.T.I.) in 1977. After 3 cycles of actinomycin-D (total dose 15 mg) a second-look laparotomy revealed progression of the disease and bulky retroperitoneal and liver metastases. When he was seen by us he also had lung metastases and impaired renal function due to left ureteric obstruction by a tumour. Reduced (75%) doses of C.D.D.P. and V.L.B. dosages were given; frusemide was also given because of fluid retention during the first treatment cycle. 3 days after discharge he was re-admitted because of septic shock (*Enterebacter ærogenes*) which had been present at least 24 h. Despite intensive treatment, the patient died of irreversible shock. At necropsy, the lung metastases consisted solely of necrotic and fibrotic tissue, but viable tumour tissue was present in the retroperitoneal lymph-nodes.

Case 3

A 52-year-old man had a right-sided orchiectomy (M.T.U.) and radiotherapy (4600 rad) to the regional lymph-nodes in 1977. 4 months later, he received chemotherapy for lung metastases. He also had hypertension. Because of his age and previous radiotherapy, the dose of C.D.D.P. and V.L.B. was reduced by 25%. The lesions regressed, but the first treatment cycle was complicated by severe myelosuppression, impairment of renal function, paralytic ileus, and sepsis (a temperature of 39·8°C but negative blood-cultures). The patient had myalgia, dizziness, and lethargy. He improved with co-trimoxazole, carbenicillin, and red-cell and platelet transfusions, but lost 13 kg in the next 2 months. Despite further reduction of V.L.B. to 50% of the initial dose, the second, third, and fourth treatment cycles were all complicated by severe granulocytopenia, thrombocytopenia, and raised serum-creatinine levels. Hearing deteriorated. 1 week after the completion of remission-induction therapy, the patient was readmitted with agranulocytic

sepsis, pneumonia in the left lower lobe, hypocalcæmia and hypomagnesæmia (with tetany), and renal function impairment. He was given amikacin and carbenicillin for a day, then placed on intravenous co-trimoxazole He became afebrile and the chest X-ray abnormalities disappeared, but he had to be haemodialysed because of renal failure. He began to have melæna, which persisted despite repeated platelet transfusions. He died of a fatal arrhythmia due to myocardial infarction while on haemodialysis. At necropsy, all metastases had disappeared; there were also a myocardial scar and signs of an adjacent infarction, severely arteriosclerotic coronary arteries, renal tubular necrosis, and two gastric ulcers.

Other Cases

Some of our patient had other side effects—inability to taste, possibly due to zinc deficiency (7 patients), foul taste (9 patients), body odour (7 patients), and transient hoarseness (3 patients). All patients had nausea and vomiting, which seems to respond to metoclopramide.[39] Weight-loss ranged from 5–13 kg in our patients at the end of remission-induction treatment. Although clinical neurotoxicity was seen in 27 patients, it is not clear whether it is due to V.L.B. or to C.D.D.P. Neurotoxicity affected only the sensory system except in 1 patient. 2 patients had C.D.D.P. allergy while on maintenance therapy; 1 had recurrent dermatitis after each C.D.D.P. administration, and the other had generalised urticaria followed by erythema and facial œdema. In both patients C.D.D.P. was withdrawn.

Discussion

There is agreement on the treatment of testicular seminomas, but none on that of non-seminoma. Although it is now widely accepted that stage III (metastases above the diaphragm or in abdominal visceral organs) should be treated with chemotherapy, some centres still irradiate solitary metastatic lung tumour. The treatment of stage II (metastases in the retroperitoneal lymph-nodes, with or without extension into the adjacent areolar tissue) is even more of a problem, since there is no clear-cut information on the effect of retroperitoneal lymph-node dissection, radiotherapy, or adjuvant chemotherapy. Factors that may influence the choice of treatment are the histological type of the tumour, the tumour load, and the presence of the tumour markers such as β-H.C.G. and A.F.P.[2,3,40–43] The lack of agreement on the treatment of choice explains why those patients who had been previously treated had received different treatments. The remission-rate, although lower than that in Einhorn's series,[24] was high, particularly when the advanced stage of the disease in most of our patients is considered. Although the numbers are small, chemotherapy seems less effective in trophoblastic teratoma.

In September, 1978, Einhorn reported that 27 out of 33 complete responders remained in complete remission for 26–49 months.[26] Patients who remain in complete remission for 2 years have been shown to have a life expectancy almost as good as that of age-matched healthy controls.[1,5] In Einhorn's series more than 50% of the original 47 patients treated with C.D.D.P., V.L.B. and B.L.M. have been in complete remission for more than 2 years and are probably cured. In our series it is still too early to assess long-term survival, since only 3 patients have completed 2 years of maintenance therapy.

Exploratory surgery has helped in the assessment of complete remission, since necrosis and fibrosis or benign differentiation can be detected only by histological examination especially in cases with residual tumour tissue in the abdomen.

The remission-induction regimen with C.D.D.P., V.L.B., and B.L.M. was very toxic, the most important side-effects being agranulocytic sepsis, renal failure, and B.L.M. lung fibrosis. The experience with case 1 shows that anuria due to this combination of drugs may be reversible and should be treated by hæmodialysis. The complication can usually be avoided by prehydration and forced diuresis, either by mannitol or saline.

Despite normal serum-creatinine levels, many patients become moderately hyponatræmic and hypokalæmic. Although we did not monitor serum-levels of magnesium, calcium, or trace metals in all of our patients, we found evidence of decreased levels in a number of them, possibly due to subclinical tubular damage. Monitoring for and supplementing these deficiencies are essential. Since the renal tubules are made vulnerable by C.D.D.P., the use of the potentially nephrotoxic antibiotics, gentamicin and cephalothin,[44] and of the diuretic, frusemide,[45,46] should be avoided. If doses of co-trimoxazole are adjusted according to serum-levels of trimethoprim and sulphamethoxazole no deterioration of renal function will occur.[47] One of the participating centres (Groningen) has used cephradine without observing nephrotoxicity. If the diuresis is less than 1 litre/6 h, a bolus of mannitol should be given. Our findings indicate that severe toxicity is more likely to occur in patients who have had previous radiotherapy and/or chemotherapy, those who are in an advanced stage of the disease, and possibly also those who are elderly. In such cases the V.L.B. dosage should be reduced by 25–50%. This was not done at the time in many of the cases in this series, but it is our present approach. Supportive measures such as intestinal decontamination, intravenous hyperalimentation, and prophylactic co-trimoxazole (which has been shown to be effective in acute leukæmia[48]) may also help to prevent serious complications, but these need further investigation.

One of the centres (Utrecht) discharges patients on the day after the completion of a treatment cycle, but only after accurate instruction, which is very important in preventing delays in re-admission if fever develops at home.

It has recently become known that B.L.M.-treated patients have an increased risk of oxygen toxicity.[49,50] This explains the rapid progression of the respiratory insufficiency in patient no. 1. In cases with respiratory insufficiency, oxygen therapy should be used with caution. During and after surgical procedures, the oxygen flow-rate should not exceed 20–25%. B.L.M. should be withdrawn when creatinine-clearance falls below 50 ml/min. because drug elimination is then severely impaired[51]—which is what probably happened in patient 1 on day 8 of the second treatment cycle.

C.D.D.P. allergy may become a commoner problem as this drug becomes used in prolonged maintenance treatment. We have been using C.D.D.P. and V.L.B. maintenance chemotherapy for more than a year now without coming across impairment of the renal function.

In view of the small numbers of patients with non-seminomatous testicular cancer and the relatively high

proportion of severe complications, we conclude that these patients should be treated in centres experienced in the complicated management of this often curable disease.

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REFERENCES

1 MacKay, E. N., Sellers, A. H. *Can. med. Ass. J.* 1966, **94,** 889.
2. Pugh, R. C. B. Pathology of the Testis, p. 139. Oxford, 1976.
3 Dixon, F. J., Moore, R. A. *Cancer*, 1953, **69,** 427.
4. Friedman, M., Purkayastha, M. C. *Am. J. Roentgen.* 1960, **83,** 25.
5. Maier, J. G., Sulak, M. H. *Cancer*, 1973, **32,** 1217.
6. Li, M. C., Whitmore, W. F., Golbey, R., Grabstald, H. *J. Am. med. Ass.* 1960, **147,** 145.
7. Li, M. C. *Med. Clins N. Am.* 1966, **45,** 661.
8. Mackenzie, A. R. *Cancer*, 1966, **19,** 1369.
9. Mackenzie, A. R. *J. Urol.* 1967, **98,** 116.
10. Samuels, M. L., Holoye, P. Y., Johnson, D. E. *Cancer*, 1975, **36,** 318.
11. Samuels, M. L., Johnson, D. E., Holoye, P. Y. *Cancer Chemother. Rep.* 1975, **59,** 563.
12. Samuels, M. L., Lanzotti, V. J., Holoye, P. Y., Eamonn Boyle, L., Smith, T. L., Johnson, D. E. *Cancer Treat. Rev.* 1976, **3,** 185.
13. Rosenberg, B., v. Camp, L., Krigas, Th. *Nature*, 1965, **205,** 698.
14 Rosenberg, B. *Naturwissenschaften*, 1973, **60,** 399.
15. Rosenberg, B. *Cancer Chemother. Rep.* 1975, **59,** 589.
16. Howle, J. A., Gale, G. R. *Biochem. Pharmac.* 1970, **19,** 2757.
17. Harder, H. C., Smith, R. G., Leroy, A. F. *Cancer Res.* 1976, **36,** 3821.
18. Harder, H. C., Smith, R. G., Leroy, A. F. *Proc. Am. Ass. Cancer Res.* 1976, **80,** 317
19. Rossof, A. H., Slayton, R. E., Perlia, C. P. *Cancer*, 1972, **30,** 1451.
20. Higby, D. J., Wallace, H. J., Albert, D. J., Holland, J. F. *ibid.* 1974, **33,** 1219.
21. Hill, J. M., Loeb, E., MacLellan, A., Hill, N. O., Khan, A., King, J. J. *Cancer Chemother. Rep.* 1975, **59,** 647.
22. Higby, D. J., Wallace, H. J., Albert, D. Holland, J. F. *J. Urol.* 1974, **112,** 100.
23. Einhorn, L. H., Furnas, B. E., Powell, N. *Proc. Am. Soc. clin. Oncol.* 1976, p. 240.
24 Einhorn, L. H., Donohue, J. *Ann. intern. Med.* 1977, **87,** 293.
25. Einhorn, L. H. *Proc. Am. Soc. clin. Oncol.*, 1978, p. 308.
26. Einhorn, L. H. National Cancer Institute Symposium on Platinum Coordination Comp, Washington, 1978.
27. Cvitkovic, E., Hayes, D., Golbey, R. *Proc. Am. Soc. clin. Oncol.* 1976, p. 296.
28. Cvitkovic, E., Cheng, E., Whitmore, W. F., Golbey, R. B. *ibid.* 1977, p. 324.
29. Merrin, C. Takita, H., Weber, R., Waysman, Z., Baumgartner, G., Murphy, G. P *Cancer* 1976, **37,** 20.
30. Merrin, C. *Proc. Am. Ass. Cancer Res.* 1976, p. 243.
31. Livingston, R. B., Bodey, G. P., Gottlieb, J. A. *Cancer Chemother. Rep.* 1973, **57,** 219.
32. Barranco, S. C., Humphrey, R. M. *Cancer Res.* 1971, **31,** 1218.
33. Hardaker, W. T., Stone, R. A., McCoy, R. *Cancer*, 1974, **34,** 1030.
34. Piel, I. J., Perlia, C. P. *Cancer Chemother. Rep.* 1975, **59,** 995.
35 Chary, K. K., Higby, D. J., Henderson, E. S., Swinerton, K. D. *Cancer Treat. Rep.* 1977, **61,** 367.
36. Cvitkovic, E., Spaulding, J., Bethune, V., Martin, J., Whitmore, W. F. *Cancer*, 1977, **39,** 1357.
37. Hayes, D. M., Cvitkovic, E., Golbey, R. B., Scheiner, E., Helson, L., Krakoff, I. H. *ibid.* p. 1372.
38. Ward, J. M., Grabin, M. E., Leroy, A. F., Young, D. M. *Cancer Treat. Rep.* 1977, **61,** 375.
39. Kahn, T., Elias, E. G., Mason, G. R. *ibid*, 1978, **62,** 1107.
40 Williams, C. *Cancer Treat. Rev.* 1977, **4,** 275.
41 Scardino, P T., Cox, D. H., Waldmann, T. A., McIntire, K. R., Mittemeyer, B., Javadpour, N. *J. Urol.* 1977, **118,** 994.
42 Javadpour, N., Scardino, P. T. *Urology*, 1977, **9,** 377.
43 LeFevre, R. E., Levin, H. S., Banowsky, L. H., Stewart, B. H., Schraffon, R A., Hewitt, C. B. *Urology*, 1975, **6,** 588.
44 Gonzalez-Vitale, J. C., Hayes, D. M., Cvitkovic, E., Sternberg, S. S. *Cancer Treat. Rep.* 1978, **62,** 693.
45 Fillastre, J. P., Laumonier, R., Humbert, G., Dubois, D., Metayer, J., Delpeck, A., Leroy, J., Robert, M. *Br. med. J.* 1973, ii, 396.
46 Lawson, D. H., MacAdam, R. F., Singh, H., Gauras, H., Hartz, S., Turubull, D., Linton, A. *Am. J. infect. Dis.* 1972, **126,** 593.
47 Tasker, P. R. W., MacGregor, G. A., de Wardener, H., Thomas, R. D., Jones, W. F. *Lancet*, 1975, i, 1216.
48 Enno, A., Catovsky, D., Darrell, J., Goldmann, J. M., Hows, J., Galton, D A G. *Lancet*, 1978, ii, 395.
49 Goldiner, P. L., Howland, W. S., Carlon, G. C. *Intensive Care Med.* 1977, **3,** 355
50 Goldiner, P. L., Carlon, G. C., Cvitkovic, E., Schweitzer, O., Howland, W. *Br med. J* (in the press).
51 Crooke, S. T., Comis, R. L., Einhorn, L. H., Strong, J. E. Broughton, A., Prestayko, A. W. *Cancer Treat. Rep.* 1977, **61,** 1631.

CURE OF APLASTIC ANÆMIA IN PAROXYSMAL NOCTURNAL HÆMOGLOBINURIA BY MARROW TRANSFUSION FROM IDENTICAL TWIN: FAILURE OF PERIPHERAL-LEUCOCYTE TRANSFUSION TO CORRECT MARROW APLASIA

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Summary The ability of syngeneic peripheral leucocytes to cure marrow aplasia was tested in a patient with paroxysmal nocturnal hæmoglobinuria (P.N.H.). Transfusion of $7{\cdot}1\times10^{10}$ white cells obtained by leucopheresis from an identical-twin donor, providing $3{\cdot}4\times10^{4}$ myeloid progenitors (C.F.U.-C)/kg, failed to improve marrow function within two months. In contrast, transfusion of $1{\cdot}3\times10^{10}$ nucleated bone-marrow cells, representing $6{\cdot}4\times10^{4}$ C.F.U.-C/kg, from the same donor resulted in prompt bone-marrow recovery. These observations support the hypothesis that aplastic anæmia in P.N.H. is a stem-cell defect that may be corrected by the simple infusion of relatively small numbers of normal bone-marrow cells. They also seem to indicate a distinct advantage of marrow cells over peripheral-blood mononuclear cells in their ability to correct marrow aplasia.

Introduction

IN mice[1] and dogs[2–4] aplastic bone-marrow can be repopulated by transfusion of pluripotential stem-cells obtained from peripheral blood. In man, the collection of large numbers of circulating normal leucocytes has been made possible by improvements in leucopheresis techniques. The feasibility of marrow repopulation by the transfusion of peripheral leucocytes is therefore of considerable practical, as well as theoretical, interest.

Referral of a patient with paroxysmal nocturnal hæmoglobinuria (P.N.H.) and refractory bone-marrow failure who had a hæmatologically normal identical-twin sister gave us the opportunity to find out whether pluripotent hæmopoietic stem-cells circulate in man and are capable of repopulating an empty bone-marrow. P.N.H. is a stem-cell disease that occasionally results in frank aplastic anæmia.[5] A previous report indicated that marrow aplasia in a patient with P.N.H. could be overcome with the infusion of relatively small numbers of normal bone-marrow cells from an identical twin.[6] We have tested the ability of syngeneic peripheral leucocytes to cure marrow aplasia resulting from a stem-cell defect.

Case-report

A 24-year-old white woman was well until April, 1978, when she developed increasing dyspnœa, fatigue, and pallor. In July, 1978, she entered hospital with a hæmoglobin level of 5·8 g/dl and white blood-cell (W.B.C.) count 1800/mm³ with 35% neutrophils and 23 000/mm³ platelets. The reticulocyte-count was 2·3%. Coombs' test was negative. A sugar-water hæmolysis test, Ham test, and hæmosiderin staining of the urine sediment were positive. Serum iron and iron-binding capacity were normal. Leucocyte-alkaline-phosphatase score