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Azathioprine in Myeloproliferative and Autoimmune Disorders

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There have been, in the last few years, some reports dealing with the chemical and biological properties of a new purine derivative, 6 [(1-methyl-4-nitro-5-imidazolyl)thio] purine.

This compound is capable of slowly releasing a purine analogue having an antimetabolic effect upon tumour tissue; the drug is in fact split to 6-mercaptopurine by alkali and sulphydryl compounds (Bresnik, 1959; Elion *et al.*, 1961).

The experiments with different animals gave variable toxicity, either immediate or delayed, depending on the dose and frequency of administration. With regard to the antitumoral activity in animals, marked inhibition of numerous experimental neoplasias and cytotoxic effects upon KB cells in tissue cultures were noted (Clarke *et al.*, 1958; Elion *et al.*, 1961).

In a clinical trial, on patients with leukaemia, Rundles *et al.* (1961), showed first of all a good tolerance of azathioprine when given by mouth in doses between 2 and 3 mg/Kg daily.

This report presents additional data based on clinical trials recently performed on patients with acute leukaemia, acute relapsing phase of chronic myelocytic leukaemia and on a group of patients affected by disorders which have probably an autoimmune pathogenesis. In the treatment of collagen diseases cytostatic drugs as well as steroid therapy in fact have recently been used (Bollag, 1963; Demis *et al.*, 1964; MacKay and Wood, 1963; Richmond *et al.*, 1963; Rundles *et al.*, 1961). The treatment is founded on the assumption that at the base of such diseases there is an autoimmune process. It is notable that cytostatic compounds inhibit immune reactions connected with humoral as well as cellular hypersensitivity and suppress antibody formation (Hitchings and Elion, 1963; Levin *et al.*, 1964). Experiments in animals could demonstrate the inhibition of immune reactions in case of transplantation of tissues and organs (Calne and Murray, 1961).

A clinical trial in patients suffering from collagen diseases, treated with cytostatic compounds (6-mercaptopurine and azathioprine) has recently been published by Lorenzen and Videbaek (1965).

The results reported by these Authors seem generally satisfactory.

Material and methods

Our case file includes 17 patients affected by acute leukaemia or the acute relapsing phase of chronic myelocytic leukaemia and 6 patients affected by autoimmune diseases. All patients were treated during their hospitalization in the Medical Clinic of the University of Milan and then, after remission, were followed-up in the Out-patient Department of the Institute.

a) Dosage and treatment

The drug was given orally at different doses and courses. In the leukaemic patients the initial dose was from 100 to 300 mg per day, but when remission was obtained the usual dose ranged from 50 to 110 mg per day.

Antibiotics, blood transfusions and corticoids were given whenever necessary. Once remission was obtained, the steroid dosage was reduced and then discontinued. Only the treatment with azathioprine was continued.

In the patients suffering from autoimmune diseases the compound was generally given for a long time, with a dosage ranging from 50 to 100 mg per day.

Most of these patients often received an associated treatment with steroids, or more rarely courses of steroids alone. The dosage will be recorded for each case.

b) Side-effects

Severe side-effects were not observed. The only significant side-effects were transient leukopenia and trombocytopenia. In only two cases (N. 7 and 12) it was advisable to discontinue temporarily the treatment for the occurrence of a severe leukopenia. In case N. 7 the dose was then adjusted according to the sensitivity so that the leukocyte count could be fairly well maintained. Also in the patient affected by ulcerative colitis severe leucopenia occurred during the treatment.

Results

a) Myeloproliferative disorders

The clinical results of patients with acute leukaemia or the acute relapsing phase of chronic myelocytic leukaemia are shown in Tab. 1.

In the table are reported the hematological picture, the length of treatment, total dose of Imuran received and the survival period from the beginning of therapy.

In 14 cases of acute leukaemia, with regard to the therapeutic activity, 6 were apparently not influenced (N. 3, 4, 6, 11, 13 and 14).

4 of the cases (N. 4, 6, 11 and 13) followed a fast downhill course with death in a few days. It is unlikely that the compound was given for a sufficient length of time to have any antimetabolic effect whatsoever.

In other 2 cases of this group (N. 3 and 14) the treatment was continued for 21

Tab. 1. Patients with acute myelogenous leukaemia and the acute relapsing phase of chronic myelocytic leukaemia

Case	Sex	Age	Diagnosis	Initial hematologic study					Imuran		Survival from beginning of treatment (in months)	
				R.B.C. $\times 10^6$	Hb %	W.B.C. $\times 10^3$	Blast cells %	Platelets $\times 10^3$	Duration of treatment (in months)	Total dose mg		
1 C.B.	♀	16	Acute myeloblastic leukaemia	0.8	13	2.8	82	16	28	37.50	30	(died)
2 B.G.	♀	34	Acute stem cell leukaemia	2.1	42	6.4	30	18	2.5	4.80	7	(not followed-up)
3* P.F.	♀	23	Acute myeloblastic leukaemia	2.2	44	6.5	70	28	0.7	2.00	0.7	(died)
4* R.P.	♀	70	Acute stem cell leukaemia	2	45	126	98	18	0.3	1.80	0.3	(died)
5 G.P.	♀	61	Acute leukaemia	2.6	42	5.1	45	90	2	4.65	2	(died)
6* C.A.	♀	33	Acute stem cell leukaemia	2.8	52	186	95	48	0.2	1.40	0.2	(died)
7 R.P.	♀	52	Acute monocytic leukaemia	2.3	48	16.8	93	70	7.3	9.75	7.3	(died)
8 C.R.	♀	57	Acute leukaemia	3.5	72	12	43	30	4	4.35	4	(not followed-up)
9 C.A.M.	♀	58	Acute leukaemia	4	82	10	88	190	16	21.35	16	
10 V.L.	♀	53	Acute myeloblastic leukaemia	2.4	47	26	91	75	7.5	7.25	7.5	(died)
11* P.P.	♀	73	Acute monocytic leukaemia	2.3	56	2.8	95	40	0.5	3.2	0.5	(died)
12 D.F.	♀	15	Acute lymphoblastic leukaemia	3.9	85	122	92	10	2.6	9.7	2.6	(died)
13* R.C.	♀	68	Acute myeloblastic leukaemia	3.2	64	11.1	51	40	0.4	2.2	0.4	(died)
14* G.A.	♀	14	Acute myeloblastic leukaemia	3.1	72	4.6	58	54	2	8.7	2.5	(died)
15 B.M.	♂	42	Acute relapsing phase of C.M.L.	2	40	11.6	80	60	12	28.45	12	(died)
16 M.A.	♂	24	Acute relapsing phase of C.M.L.	2.6	45	290	12	70	3	8.7	3	(died
17 M.G.	♂	35	Acute relapsing phase of C.M.L.	3.4	60	33	71	70	1.2	6.8	2.6	(died)

* patient did not respond to therapy.

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days and 2 months respectively, but the combined treatment with azathioprine and steroids, even though given for a sufficient time, did not change the course of the illness.

In the remaining 8 cases remissions were obtained: in cases N. 7 and 9 the remission was complete; in the other 6 cases both clinical and haematological pictures showed some improvement. The duration of such partial remission ranged from 2 to 28 months.

One patient with a remission of 16 months is still in medical control.

The number of remissions obtained in patients suffering from acute leukaemia of the present case file treated with azathioprine and prednisone was 57%.

This result is even more important if we consider that we are dealing with a case file of adult patients.

In 3 cases of chronic myelocytic leukaemia in acute relapsing phase, 1 did not respond to therapy (N. 17), the other 2 (N. 15 and 16) showed a remission respectively of 1 year and 3 months.

As far as therapeutic effects of azathioprine in leukaemic patients are concerned, the present data do not show an homogeneous pattern, particularly in cases of acute leukaemia.

A complete lack of success observed in 4 cases, with patients dying in a very brief time, shows that even this drug cannot influence some cases of acute leukaemia. It must also be noted that it is difficult to forecast the evolution of the disease according to the various clinical parameters (Gehan, 1960).

In this group of patients, 1 is still alive and controlled in the Out-patient Department, 2 were lost from observation when they were still in good condition, the others are dead. The median survival cannot therefore be exactly determined, but it is definitely above 7 months. The length of survival of these patients was compared to that of a similar group of patients affected by acute leukaemia treated in the same period of time with 6-mercaptopurine at the dosage of 2.5 mg/Kg daily (Fig. 1).

It can be seen that the effects of the two compounds, as measured by such features, are very similar.

The remissions obtained in 2 out of 3 cases of chronic myelocytic leukaemia in acute relapsing phase are more promising considering the extreme difficulty of obtaining remissions in this phase of the disease. It is however necessary to study a larger case file to support this impression.

b) Autoimmune disorders

Before performing the clinical trials with azathioprine in some diseases probably having an autoimmune pathogenesis, an experimental trial of the effects of the drug on the transformation into blast cells of peripheral cells cultivated *in vitro* in the presence of phytohaemagglutinins (P.H.A.) was performed. According to the point of view most commonly accepted, in fact, such a process is immunological in nature, and may be stimulated by antigens other than P.H.A.

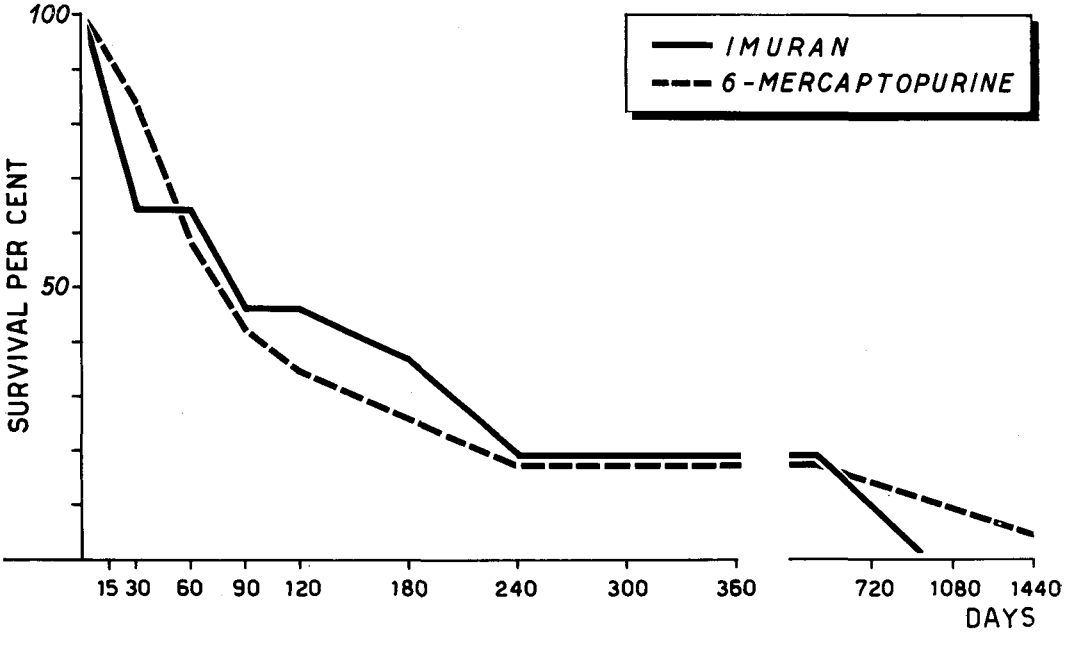

Fig. 1. Survival of patients suffering from acute leukaemia

The modification of the lymphocytic cells stimulated by different antigens would be at the basis of immune reaction of tissues, but the extent of the similarity between the blastoid cell formed *in vitro* and those formed in the immunological response *in vivo* remains to be elucidated (Elves *et al.*, 1963; Hirschhorn *et al.*, 1963).

The percentage of cells which are capable of blastic transformation was determined at different intervals of time in basic condition and after a treatment of Imuran in doses of 100 mg per day (Fig. 2).

The results show that, after the first course of therapy, some lymphocytes are still capable of blastic transformation, but such potentiality is almost totally lost after a longer period of treatment.

The implication can be drawn that an analogous treatment can break in one step or another the sequence of events which induces antibody formation against a variety of antigens (even self-antigens) in immunological disorders. On this assumption a clinical trial was performed in some diseases which may have a pathogenetic component of immunological nature.

The present case file includes 3 cases of acquired autoimmune haemolytic anaemia, 1 case of thrombotic thrombocytopenic purpure (T.T.P.), 1 case of ulcerative colitis and 1 case of nephrotic syndrome.

In Tab. 2 the clinical results of the patients affected by autoimmune haemolytic anaemia are reported.

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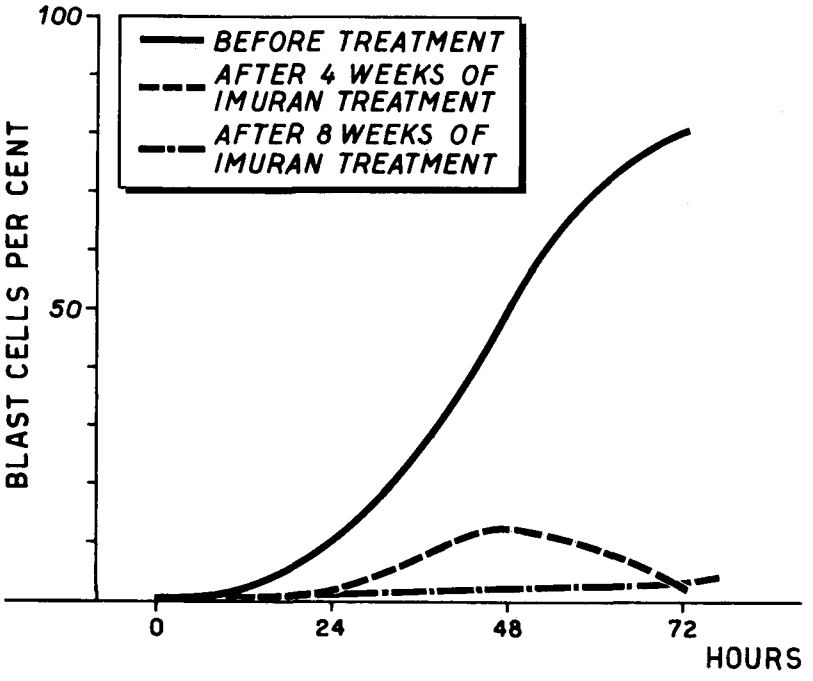

Fig. 2. In vitro cultures of human leucocytes from a patient with collagen disease (S. L. E.) in presence of P. H. A.

In 2 of such patients the syndrome occurred during the course of systemic lupus erithematosus (S.L.E.) which was treated, for a long time, with corticosteroids (N. 1 and 2).

At the beginning of treatment the patient N. 1 suffered from severe anaemia (Hb 9.6 gm/100 ml, RBC 2 800 000) and osteoporosis probably due to a long previous treatment with steroids. The azathioprine was given and prednisone discontinued; the steroid was added only to the second course of Imuran.

After 3 months of treatment the patient showed a reduced positivity of the abnormal serological findings, an improvement of anaemia and a decrease in the bone pain. Also the Coombs test became negative.

Case N. 2, at the onset of the treatment with Imuran showed severe arthralgic syndrome, anaemia (Hb 8 gm/100 ml, RBC 2 600 000), and kidney involvement. Serological tests of collagen diseases, Coombs test, either direct or indirect, and the blood serological test of syphilis were also strongly positive. Prednisone was reduced at the beginning of the Imuran treatment and then was discontinued.

The patient very soon showed some improvement of subjective symptoms, which persisted after 8 months of treatment. The Coombs test became negative and a slight correction of anaemia was obtained (Hb 9.5 gm/100 ml - RBC 3 400 000).

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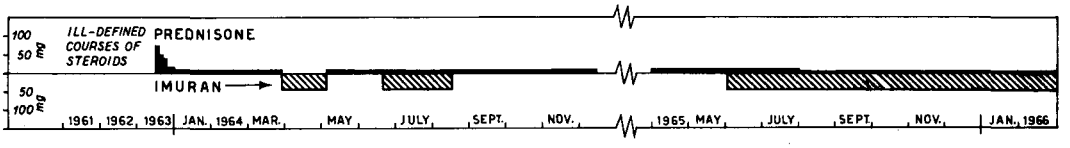
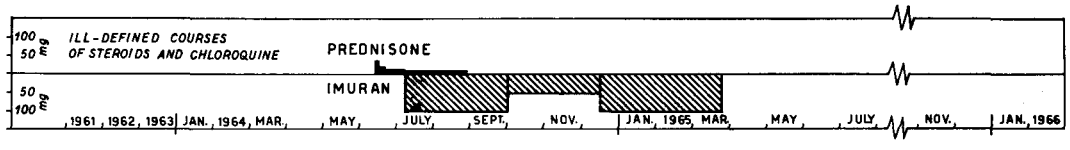
Tab. 2. Details of treatment and clinical results in patients with autoimmune haemolytic anaemia

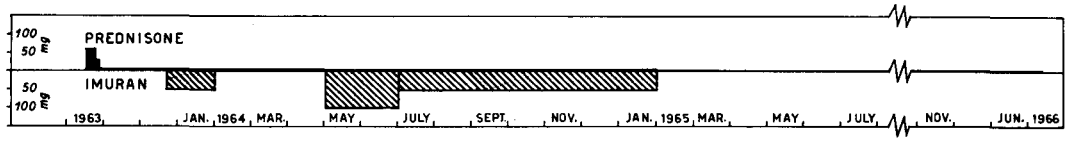
Case	Sex	Age	Diagnosis	Total dose of drug g.	Duration of treatment (in months)	Serological findings			Clinical result
							before treatment	after treatment	
1 P.E.	♀	35	S.L.E. with autoimmune haemolytic anaemia	18	11.5	E.S.R.	1th 127 2ndh. 141	1thh 65 2ndh. 78	very good
						S.T.S.	+++	+	
						L. E. cell	+++	+	
						Rose-Waler	+	— ±	
						Coombs Test			
						direct	positive	negative	
						indirect	positive	negative	



2G.V.	♀	57	S.L.E. with autoimmune haemolytic anaemia	21.2	8.5	E.S.R.	1thh. 145 2ndh. 150	1thh. 75 2ndh. 100	fair
						S.T.S.	+++	++	(the treat-
						L.E. cell	++	+	ment al-
						L.E. test	+	±	lowed the
						Rose-Waler	++	+	discontin-
						Antinuclear			uance of
						factor	+	+	steroid
						Coombs Test			therapy)
						direct	positive	negative	
						indirect	positive	negative	



3 B.L.	♀	36	acquired autoimmune haemolytic anaemia	20	11.5	E.S.R.	1thh. 164 2ndh. 166	1thh. 20 2ndh. 30	good
						S.T.S.	negative	negative	
						Coombs Test			
						direct	positive	negative	
						indirect	positive	negative	



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The patient followed-up in the Out-patients Department could not be observed after March, 1965.

Case N. 3 suffering from acquired autoimmune haemolytic anaemia was admitted during a severe haemolytic crisis (Hb 4 gm/100 ml - RBC 1 200 000). After a course of prednisone given at high doses, the blood picture showed a remarakble improvement (RBC reaching 3 500 000 with 11.2 gm of Hb).

At this point Imuran treatment was started: the Coombs test was still positive. At the same time the prednisone was reduced to 5 mg per day.

After 5 months of treatment, the Coombs test became negative and the haematologic picture became normal. After a month of treatment Imuran was stopped.

The patient now enjoys good health and follows courses of 25 mg prednisone daily for variable periods of time.

Case N. 4 was admitted to the Hospital suffering from thrombocytopenic syndrome (platelets 67 000/cu.mm), and thrombosis of the right leg.

The megakaryocitic anti-globulin test, according to Coon and Kaplan was positive.

After a course of antibiotics and prednisone, the platelet count reached 120 000/cu.mm.

At the discontinuance of prednisone a decrease in the platelet count (platelets 90 000/cu.mm) occurred. After therapy with Imuran the platelet count increased again to 130 000, and this level was maintained during the period of treatment. No purpura was present. (The patient was not followed up after the 18 May, 1964) (Fig. 3).

Case N. 5, a 26 year-old woman, suffering from ulcerative colitis from November, 1963, was admitted to the Hospital on December 1965; for a long period of time before hospitalization, the patient followed an indefined course of treatment with antibiotics (neomicin) and sulfonamides.

On January 10, 1966 the patient began treatment with Imuran, in a dosage of 100 mg on alternative days. This dosage was reduced to 50 mg after a month, as leukopenia, already present at beginning, increased.

The patient kept a variable course, alternating periods of remission and relapse, but never with severe deterioration.

Case N. 6, is a 58 year-old woman, suffering from severe nephrotic syndrome.

The initial treatment with only prednisone did not change the severe albuminuria and the clinical symptoms. Imuran was therefore associated at the dose of 200 mg daily for 11 days and was successively reduced to 100 mg per day (Fig. 4).

A slight, transient decrease of albuminuria and a temporary increase of serum proteins were observed. After that the general conditions deteriorated again, with a clinical picture as at the beginning.

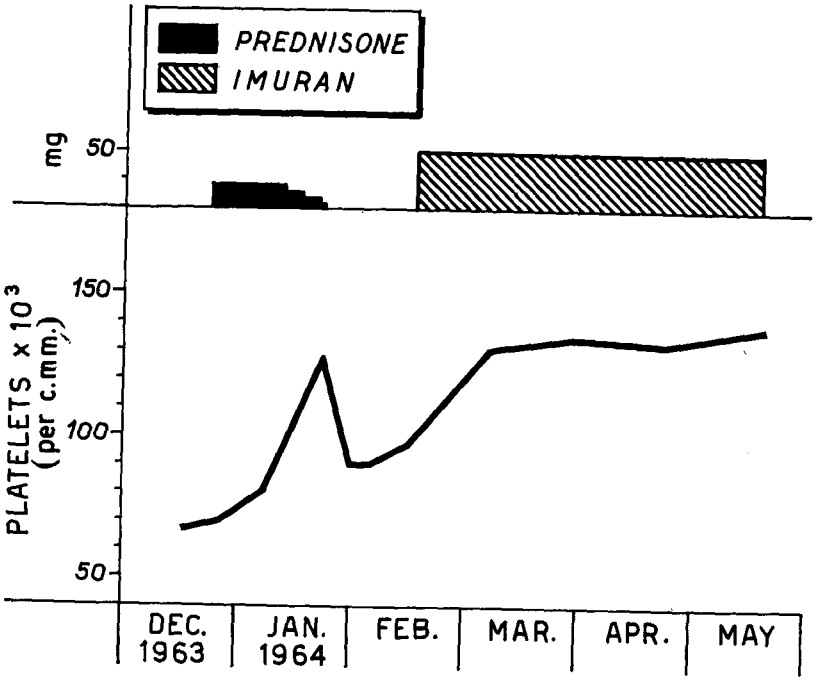

Fig. 3. Therapeutic result in a case of T. T. P.

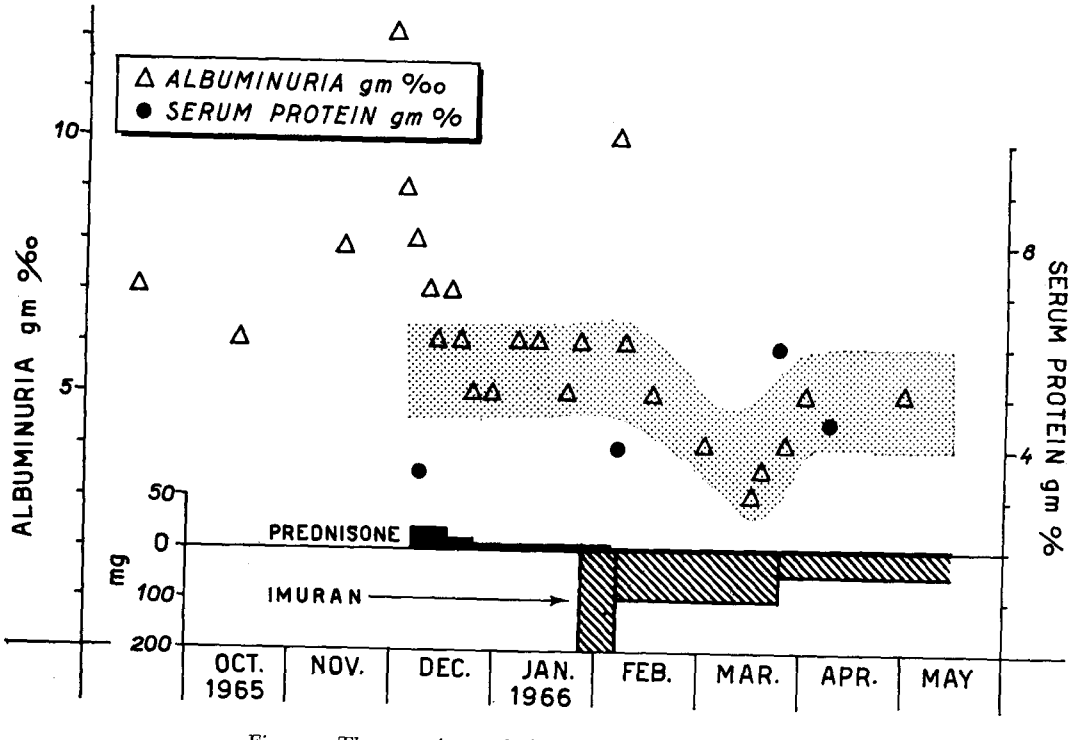

Fig. 4. Therapeutic result in a case of lipoid nephrosis

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Conclusion

The clinical results obtained in the present case file show a favorable effect of Imuran in the group of patients suffering from acute leukaemia.

Even though the median survival does not seem to differ remarkably from that observed in patients suffering from the same disorder and treated with 6-mercaptopurine, azathioprine seems to present some advantage particularly with regard to side-effects.

A fair response was also obtained in the group of patients in the acute relapsing phase of chronic myelocytic leukaemia.

As far as Imuran treatment in autoimmune disorders is concerned, the response appears favorable in the patients suffering from S.L.E. and haemolytic anaemia.

The assessment of results in the patient with ulcerative colitis is not yet possible because of the persistent variability of the whole picture.

It must however be pointed out that favorable responses in this disease were recently recorded by Bowen *et al.* (1966). The temporary benefit observed in the patients with nephrotic syndrome and thrombotic thrombocytopenic purpura still needs a careful evaluation which will be carried out in the long run. The treatment with azathioprine often allows one to discontinue the corticosteroid therapy or to reduce its dosage in patients, who after prolonged steroid treatment have presented severe side-effects.

Summary

The report presents the clinical results of treatment with Azathioprine 6 [(methyl-4-nitro-5-imidazolyl) thio] purine, in patients with acute leukaemia, the acute relapsing phase of chronic myelocytic leukaemia and in a group of patients affected by illness which have probably an autoimmune pathogenesis.

Favorable results were obtained in the group of patients suffering from acute leukaemia with a percentage of clinical and/or haematological remission of 57%.

A comparative analysis of the length of survival in these patients compared to that of a similar group of patients treated with 6-mercaptopurine is also reported.

A fair response was also obtained in the group of patients in the acute relapsing phase of C.M.L.

In the group of patients affected by diseases probably due to an immune pathogenesis, a favorable response was obtained in 2 cases of systemic lupus erithematosus with haemolytic anaemia and 1 case of acquired autoimmune haemolytic anaemia.

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RIASSUNTO

Vengono riportati i risultati clinici ottenuti in pazienti affetti da leucosi acuta, leucemia mieloide cronica in fase di riacutizzazione ed affezioni a probabile patogenesi autoimmunitaria trattati con Azatioprina (6-[(metil-4-nitro-5-imidazolil) tio] purina). Risultati favorevoli sono stati ottenuti nei pazienti affetti da leucosi acuta con percentuali di remissioni cliniche e/o ematologiche del 57%.

Viene pure riportata un'analisi comparativa della durata della sopravvivenza in tali pazienti con un gruppo analogo di pazienti affetti da leucosi acuta e trattati con 6-mercaptopurina.

Risposte discrete si sono anche avute in pazienti affetti da leucemia mieloide cronica in fase di riacutizzazione.

Nel gruppo di pazienti affetti da malattie a probabile patogenesi autoimmunitaria si sono ottenuti risultati favorevoli in due casi di lupus eritematosus sistemico con anemia emolitica ed in un caso di anemia emolitica acquisita.

RÉSUMÉ

Les Auteurs présentent les résultats cliniques obtenus sur les patients atteints de leucose aiguë, leucémie myéloïde chronique en phase aiguë terminale et affections avec une probable pathogénie autoimmune et ayant subi un traitement d'Azathioprine (6-[(methyl-4-nitro-5-imidazolyl) thio] purine).

Des résultats satisfaisants ont été obtenus chez les malades atteints de leucose aiguë avec un pourcentage de rémissions cliniques et/ou hématologique de 57%.

On rapporte également une analyse comparative de la durée de survie chez ces patients avec un groupe de patients atteints de leucose aiguë et traités par 6-mercaptopurine.

On a également obtenu d'assez bonnes réponses chez les malades atteints de leucémie myéloïde chronique en phase aiguë terminale. Parmi le groupe de patients atteints de maladies avec une probable pathogénie autoimmune on a obtenu des résultats satisfaisants dans deux cas de lupus erithematosus systémique avec une anémie hémolytique et dans un cas d'anémie hémolytique acquise.

ZUSAMMENFASSUNG

Verf. berichten über die klinischen Ergebnisse der Anwendung von Azathioprina (6-[(methyl-4-nitro-5-imidazolyl) thio] purina) bei P. mit akuter Leukose, mit chronischer, im Wiederaufflammen begriffener Myelose und mit Krankheiten, die wahrscheinlich autoimmunitären Ursprungs sind. In 57% der Fälle mit akuter Leukose konnte eine klinische und/oder haematologische Besserung erzielt werden.

Sodann wird die Überlebenszeit verglichen zwischen oben genannten P. und einer analogen Gruppe von Fällen mit akuter Leukose, welche mit 6-Mercaptopurina behandelt worden waren.

Auch die P. mit chronischer, im Wiederaufflammen begriffener Myelose sprachen ganz gut auf die Therapie an.

Unter den P. mit wahrscheinlich autoimmunitär bedingten Leiden erreichten Verf. günstige Erfolge in 2 Fällen von Lupus erythemat. system. mit Anaemia haemolytica und in 1 Fall von erworbener Anaemia haemolytica.

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