POLISH MEDICAL JOURNAL, Vol. X, No. 3/1971
(Translated from Polski Przegląd Chirurgiczny, Vol. XLII, No. 1/1970)

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BIOCHEMICAL AND HISTOLOGICAL CHANGES FOLLOWING IMURAN (AZATHIOPRINE) ADMINISTRATION

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Healthy dogs divided into two groups were administered immunosuppressive agent Imuran in scope to assess organ lesions resulting from this drug. Dogs of group 1 received low doses of Imuran, usually applied after organ transplantation. In this group Imuran was administered for a 6 month period. Dogs belonging to group 2 were administered toxic doses of Imuran, like those used in the period of acute transplant rejection. Following symptoms and lesions were noted in both these groups: loss of body weight, leukopenia, hypoalbuminemia, atrophy of lymphatic tissue and lesions in parenchymatous organs. One half of dogs of group 1 survived the experiment while all dogs of group 2 died. Histological examination revealed that alterations in kidneys and livers due to Imuran administration differed from those found in allotransplants of these organs.

Imuran belongs to immunosuppressive drugs controlling the rejection process in the transplanted organs. Prolonged administration of this drug may produce injury of bone marrow, spleen and lymph nodes, as well as agranulocytosis and liver necrosis. When administered in high doses it may induce inanition and death (1, 2, 3, 4, 5, 6, 7). The knowledge of toxic effects of Imuran, is of prime importance in differentiating between toxic and rejection changes in the transplanted organs, particularly the liver.

The following study has been undertaken to answer 2 important questions: 1) what biochemical changes are produced by Imuran administered to healthy dogs, a) at low chronic doses used routinely after transplantation, b) at high doses recommended for acute rejection, 2) what histological changes develop in parenchymatous organs after Imuran administration.

METHODS

The study was carried out in 2 groups of 9 mongrel dogs weighing 10—13·5 kg.

In group 1 of 6 dogs, Imuran was given by mouth daily for a period of 6 months, with an initial dose of 4 mg/kg/day changed later according to WBC.

The lowest dose was 1 mg/kg/day (Fig. 1, 2). Control studies carried out in all dogs included red blood cell count, white blood cell count, serum protein electrophoresis, serum alkaline phosphatase activity, blood urea level, and blood coagulation time. In all cases control biopsies of liver and popliteal lymph node were performed. In 5 dogs bone marrow was examined. In the course of Imuran administration dogs were weighed on alternate days, and WBC was done. Liver, skeletal muscle, skin and lymph node biopsies were carried out at intervals of 2—10 weeks. RBC, serum protein electrophoresis, alkaline phosphatase, blood urea level and coagulation time were determined every week. At the end of the experiment or after death of the animal before the planned 6 months follow-up period, the following organ specimens were taken for histology: liver, spleen, lymph nodes, kidneys, myocardium, skeletal muscles, brain and skin.

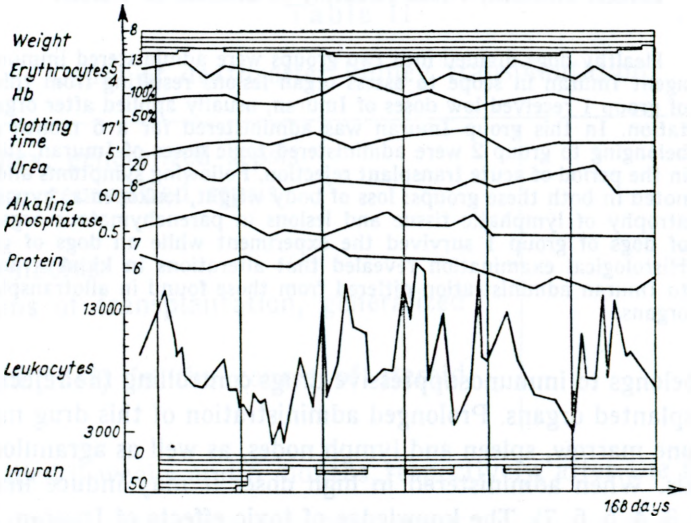

Fig. 1. Laboratory data and Imuran administration schedule in a dog of group 1 surviving the experimental period.

In group 2 of 3 dogs Imuran was given until death at a dose of 12 mg/kg/day (Fig. 3). The same studies as in group 1 were performed before, during and after Imuran administration.

RESULTS

Group 1. Out of 6 dogs in that group 3 dogs succombed after 29, 70 and 164 days of the experiment: 2 of pneumonia, and 1 of bone marrow atrophy and inanition. Three other dogs survived the 6 months period of experimentation.

1. Body weight. During the first 3 weeks of Imuran administration dogs body weight decreased by 1—2 kg, and remained at that level until death of the animal or the end of the experiment.

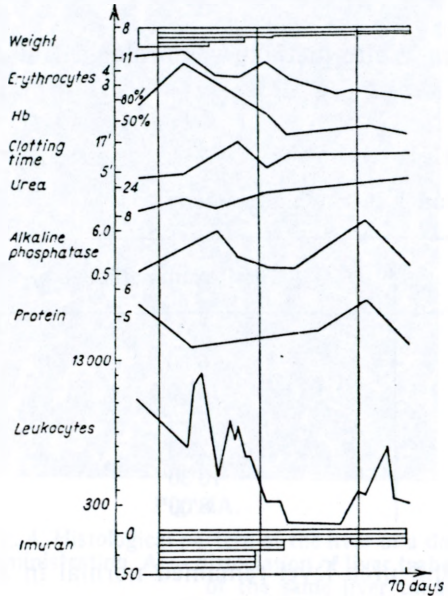

Fig. 2.

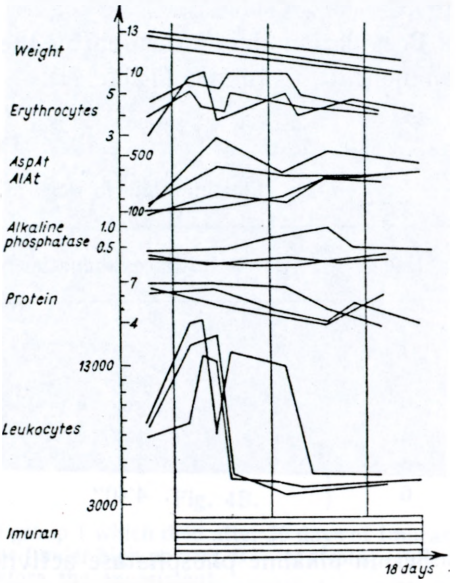

Fig. 3.

Fig. 2. Laboratory data of a dog of group 1 which died on the 70 day of experiment.
Fig. 3. Laboratory data of 3 dogs of group 2, with high Imuran dosage 12/mg/kg/day.

Table I

Initial and final levels of serum total protein and albumin

Group	Dog	Total protein in g%		Albumin in g%		Survival
		initial level	final level	initial level	final level	
I	1	7·0	5·9	45	41	died 29 days
	2	6·9	5·5	35	33	survived
	3	6·0	5·7	61	45	survived
	4	6·4	4·8	62	43	died 70 days
	5	6·8	4·1	50	35	died 164 days
	6	6·5	5·9	41	34	survived
II	1	6·8	3·8	39	31	died 16 days
	2	6·6	3·8	40	31	died 17 days
	3	6·0	3·0	52	44	died 18 days

2. Biochemical studies. Leukopenia occurred in all dogs WBC being 2,200 to 3,900 (Fig. 1, 2, 3). Red blood cell count was normal in 4 dogs, and in 2 dogs which died, it decreased from the initial $5{\cdot}17 \times 10^6$ to $2{\cdot}14 \times 10^6$, and to $3{\cdot}19 \times 10^6$ in cu mm. Hypoproteinemia was observed in all dogs. Serum protein level decreased from 6·0—7·0 g% to 4·1—5·9 g%, and albumin level from 41—62% to 32—45% (Table I).

Coagulation time measured by the Lee-White method was prolonged in all animals to 6—15 min (Table II).

Table II

Clotting time in dogs of group 1 (Lee-White method)

Dog	Initial examination	Total examination
1	2′30″	6′30″
2	4′20″	15′00″
3	3′40″	7′00″
4	1′40″	12′00″
5	3′20″	10′30″
6	4′40″	8′00″

Serum alkaline phosphatase activity and urea level remained normal in all studies and all dogs.

3. Bone marrow. Marrow smears were normal in 3 dogs which survived. In 1 dog which died on the 29th day single lymphoreticular system cells, mature lymphocytes, reticular cells and ghost cells were seen. This findings were consistent with bone marrow atrophy.

In another dog living 164 days, a transitory erythroblastic reaction with normal maturation of erythrocytes and weak reticular reaction were seen in the 4th month of experiment. A smear taken 3 days before death was normal.

4. Histological studies. In 3 dogs which survived the 6 month period of Imuran administration consecutive liver and lymph node biopsies did not reveal any abnormalities. In 3 dogs which died major morphological changes were found. In the dog living 29 days dissociation of liver trabeculae, and atrophy of lymph nodules in the spleen were seen (Fig. 4, 5). In the dog surviving 70 days apart from dissociation of liver trabeculae multiple necrotic foci with inflammatory and resorptive infiltrations were encountered in the liver, in the kidneys multiple abscesses, in the spleen atrophy of lymphoid tissue with only few preserved nodules and dilatation of sinuses, in the lymph nodes decrease in number of lymphatic nodules and dilatation of sinuses (Fig. 6). In the dog living

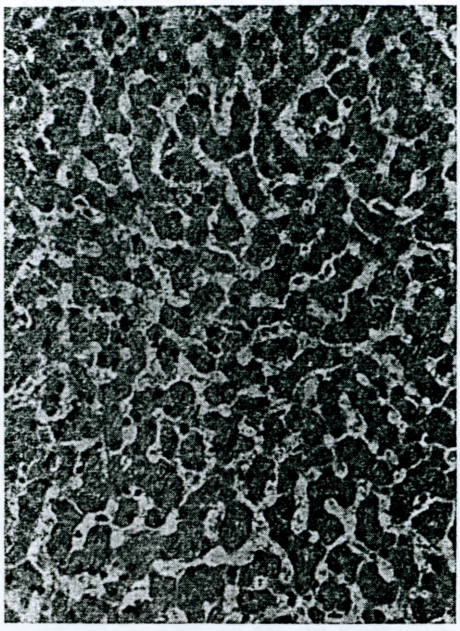

Fig. 4A.

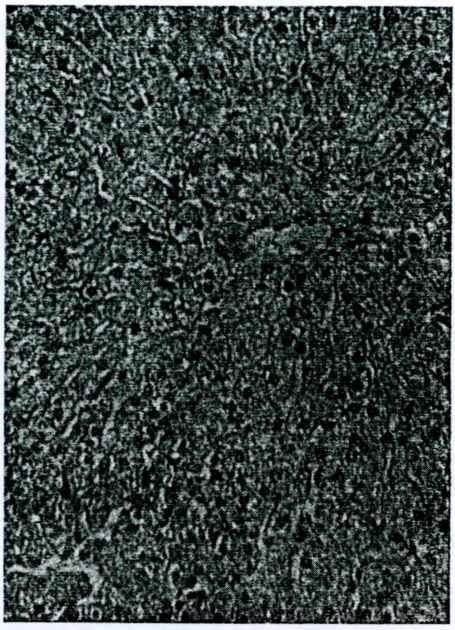

Fig. 4B.

Fig. 4. Histological pattern of the liver of a dog of group 1 which died after 29 days of Imuran administration. A — dissociation of liver trabeculae (under high power), B — control specimen of the same liver taken before the experiment.

Fig. 5. Microscopic appearance of the spleen of the dog whose liver is shown in Fig. 4. Atrophy of lymphoid tissue.

164 days there was dissociation of liver trabeculae, in the spleen-atrophy of lymphoid tissue, in the lymph nodes-atrophy of lymphatic nodules, and in the lungs-bronchopneumonia.

No changes in skeletal muscles, myocardium, brain or skin were found in that group.

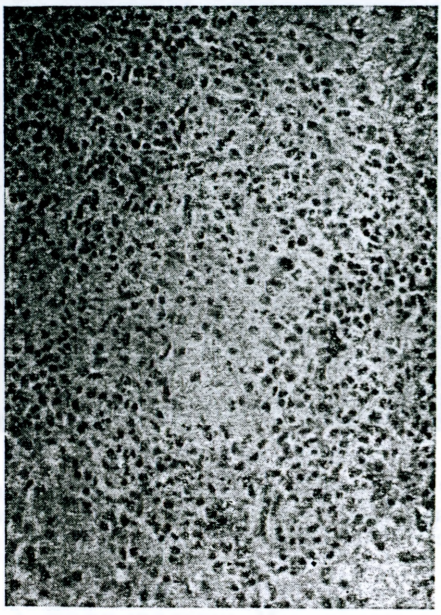

Fig. 6A.

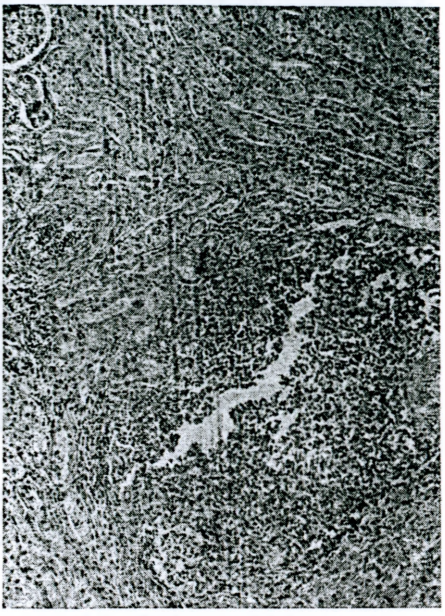

Fig. 6B.

Fig. 6. Autopsy specimens of a dog of group 1, which died after 70 days of Imuran administration. A — liver necrotic focus, B — renal abscess.

Group 2. All dogs of that group died by the end of the 3rd week (16, 17 and 18th day) of Imuran administration.

1. Body weight. Body weight los was observed in all dogs and was much higher than in group 1. Two dogs had a loss of 2 kg, one 3 kg.

2. Biochemical studies. WBC reached 14,000—16,000 in all dogs on the second day of Imuran administration, and from the 4th day on WBC tended to fall down to 4,600, 4,200 and 4,1000 per cu mm, respectively. In all dogs hypoproteinemia occurred. Serum protein level decreased on the average from 6·65 to 3·5 g% (Table I). In 2 dogs serum transaminase activity was measured. In both cases an increase in AlAt activity was found up to 380 and 440 μ, and AspAt up to 300 and 400 μ.

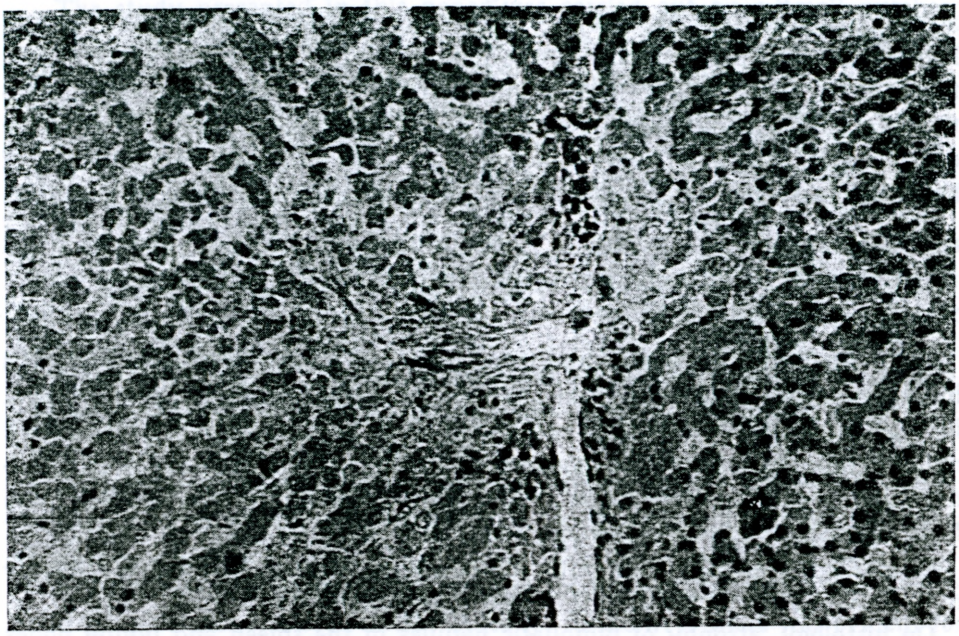

Fig. 7A.

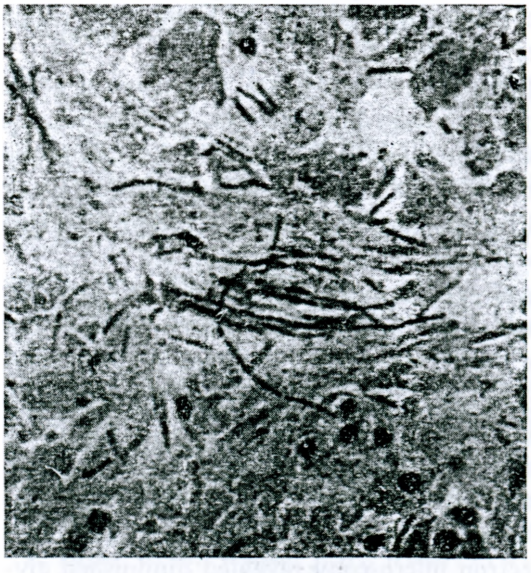

Fig. 7B.

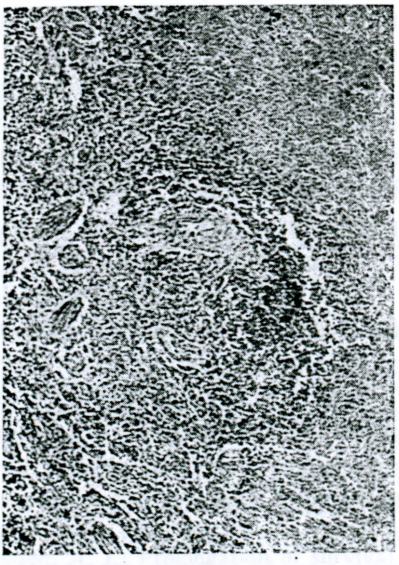

Fig. 7C.

Fig. 7. Microscopic appearance of liver and spleen of a dog of group 2 which died after 17 days of high dosage of Imuran 12 mg/kg/day. A — liver necrotic focus, B — accumulation of anaerobes in the liver (high power), C — atrophy of spleen lymphoid tissue

3. Histological studies. Histological studies revealed in the liver fluid accumulation between hepatocytes, dissociation of trabeculae, focal atrophy of hepatocytes, sharply defined necrotic foci, in the spleen-atrophy of lymphoid tissue, in the lymph nodes-decrease in number of lymphoid nodules with dilatation of medullary and marginal sinuses, in one case, a suppurative focus in the central part of the popliteal lymph node. In one of these dogs accumulation of hematoxylin-stained anaerobics was found in the necrotic focus in the liver.

DISCUSSION

In group 1 Imuran was administered to the dogs for a period of 6 months in doses routinely used after kidney or liver transplantation. Three dogs died, in 2 of them pneumonia was found at autopsy, in the third-atrophy of bone marrow. Histological studies of autopsy specimens revealed apart from pneumonia atrophy of the lymphoid tissue of spleen and lymph nodes, and in 1 case focal necrosis of liver and abscess of the kidney.

In all dogs of both groups there was evident leukopenia, serum hypoproteinemia, prolongation of clotting time and body weight loss.

On histology, atrophy of lymphatic nodules in the spleen and lymph nodes and hepatic changes of various degree were seen in all dogs. There was fluid accumulation between hepatocytes and dissociation of trabeculae of the liver in dogs of group 1 which survived the whole experimental period, as well as necrotic foci in the liver, and renal abscesses in the dog of the same group which succombed at an early stage of the experiment.

In group 2 Imuran was administered in the same doses as in case of acute rejection of the transplant. In this group all dogs died after 16—18 days because of toxic changes in parenchymatous organs, and inanition. Also in this group marked leukopenia, hypoproteinemia, increase in amino-transpherase activity and body weight loss were observed. Histologically, atrophy of lymphoid tissue of spleen and lymph nodes and focal liver necrosis were revealed.

Histological patterns seen in dogs after Imuran administration, in particular demarcating necrotic foci in the liver were different from those described for rejection process i.e. lymphocyte infiltrations and colliquative necrosis.

In studies analogical to ours, performed by *Starzl et al.* (6), healthy dogs were given Imuran in doses of 2—4 mg/kg/day for a period of 40 days. Only 1 dog survived. The authors observed in all animals body weight loss, low hematocrit, leukopenia, increase in serum aminotransferase activity, and alkaline phosphatase. Microscopically hepatic necrotic foci and atrophy of lymphoid nodules were seen in autopsy specimens. Electron microscopy revealed diminished glycogen stores in hepatocytes and dilatation of bile canaliculi.

The study presented in this paper was carried out on a small number of animals, mostly because of the very high price of Imuran. Even so the histological patterns of liver and kidney were highly reproducible, and appeared to be different from those usually seen in rejected allotransplants.

CONCLUSIONS

1. In a group of 6 normal healthy dogs Imuran was administered by mouth for a period of 6 months at chronic doses usually recommended for prevention of kidney and liver transplant rejection. Fifty percent of dogs died of pneumonia and bone marrow atrophy.
2. In another group of 3 normal dogs, Imuran was given at high doses administered usually in case of acute rejection. All animals died of toxic injury to the parenchymatous organs, and inanition.
3. Laboratory studies revealed in both groups leukopenia, hypoproteinemia, prolongation of clotting time, increase in serum aminotransferase activity and body weight loss.
4. In all dogs of both groups atrophy of lymphoid nodules in the spleen and lymph nodes was seen microscopically. In the liver there was evident focal necrosis in the dogs which succombed.

The authors are indebted to Dr *B. Mornington* from Baroughs Company in London for providing Imuran.

Translated by W. Olszewski, M. D.

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