Immunosuppressive effect of A.CA placenta fractions in A.CA recipients of split A/Sn heart transplants

Treatment	No. of transplants	Mean survival and range (days)
None	8	11.3 (9–13)
Placenta homogenate[a] (A.CA × A/Sn)	8	13.5 (10–20)
Placenta pellet[b] (A.CA × A/Sn)	6	11.0 (10–12)
Placenta supernatant[a] (A.CA × A/Sn)	6	13.0 (11–15)
Placenta supernatant concentrated[a] (A.CA × A/Sn)	9	15.3 (13–17)
Placenta supernatant concentrated[a] (A.CA × A.CA)	5	11.5 (10–12)
Serum from pregnant A.CA (A.CA × A/Sn[c])	11	10.3 (7–13)
Amniotic fluid from pregnant A.CA (A.CA × A/Sn[d])	9	11.4 (9–14)

[a] 0.3 ml given i.p. on every 2nd day after transplantation starting on day 0. [b] 0.15 ml given i.p. on every 2nd day after transplantation starting on day 0. [c] 0.15 ml of serum given i.p. on days 0, 6 and 9 after transplantation. [d] 0.15 ml of amniotic fluid given i.p. on days 0, 6 and 12 after transplantation.

A.CA recipients received 0.15 ml of the resuspended pellet or 0.3 ml of the concentrated supernatants (24 mg protein/ml) i.p. on every 2nd day after transplantation, starting on day 0.

Results and discussion. Placenta supernatant, particularly after concentration, significantly ($t_{(16)} = 5.51^{XXX}$) prolonged the survival of strongly H-2 incompatible split A/Sn heart transplants (Table). In contrast, neither placenta supernatant (24 mg protein/ml) from A.CA females mated with A.CA males, nor amniotic fluid (12 mg protein/ml) or serum from A.CA females mated with A/Sn males, had any significant immunosuppressive effect.

The negative results with amniotic fluid and placenta supernatants from females of the A.CA × A.CA mating speak against α-fetoprotein[19] as the major immunosuppressive factor. Neither do the latter results support the idea that placental glycoprotein hormones[11,13–16] are responsible for the effect. Other possible factors which could explain the immunosuppressive effect are maternal serum-derived α-globulins[17] or antibodies[4]. The fact that only placenta supernatant from A.CA mice mated with A/Sn males had distinct immunosuppressive effect is compatible with an effect mediated by antibodies or antigen-antibody complexes; but further experiments are necessary in order to identify the active principle.

[19] S. S. OGRA, R. A. MURGITA and T. B. TOMASI, JR., Immun. Commun. *3*, 497 (1974).

Effect of an Antiandrogen on the Lymphoid System*

MARTA VOJTÍŠKOVÁ, MARTA POLÁČKOVÁ and V. VIKLICKÝ

Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Budějovická 1083, Praha 4 (Czechoslovakia), 15 March 1976.

Summary. A side-effect of the administration of cyproterone acetate, an antiandrogenic steroid, to newborn, juvenile or adult male mice (in doses comparable to those used clinically) was found in a marked reduction of the white pulp of the spleen and reduced weight or even absence of the thymus.

The antiandrogenic steroid, cyproterone acetate (6-chloro-17-hydroxy-1α,2α-methylene-pregna-4,6-dien-3,20 dione acetate, Androcur, Schering AG, Berlin) is being used in human medicine when the production of endogenous testosterone is to be inhibited (sexual de viations such as hypersexuality, benign prostatic hyperplasia, prostatic cancer)[1–5].

We used cyproterone acetate (CA) in some experimental studies aimed at specifically inhibiting certain cellular antigens of which the expression turned out to be to some extent androgen-dependent[6,7]. CA was given s.c. to newborn, juvenile or adult male mice for 4 or 6 weeks or only as a single dose (Table). The long-term dosage was calculated (on body weight basis) from the doses reported to be administered per os in man. That their biological effect may be equivalent seems to be indicated by a similar picture of spermatogenesis which was not completely disturbed, only the production of sperm being markedly inhibited.

The body weight was reduced only in the groups where the treatment was started in newborn or juvenile males. Spermatogenesis was inhibited in all groups and testes weight reduced in most of them. A reduced spleen weight was found only when the treatment was started neonatally, but the microstructure of the organ was affected in all groups; there was always an increased granulopoiesis and erythropoiesis in the red pulp, whereas the white pulp was reduced and occasionally almost completely lacking. In the thymus, the change concerned weight and mostly also morphology irrespective of the age at which the treatment was started and the dose administered. The organ was sometimes hard to detect, even histologically. The striking reduction of size was in some cases accompanied by so drastic a depletion of lymphocytes that the cortex and medulla were virtually indistinguishable. Also frequently observed was dilatation of blood vessels in the medulla. Following a single administration of CA, particularly in the higher dose range, the normal structure of the thymus was sometimes reversed in that the medulla contained more lymphocytes than the depleted

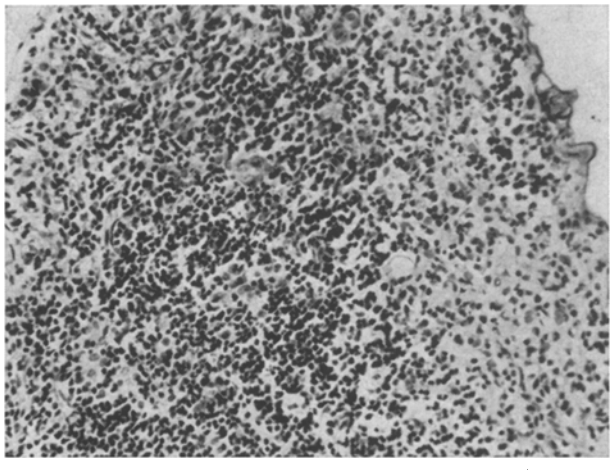
Direct and side-effects of cyproterone acetate on C57BL/6 male mice

Mode of administration		Mean weight (± standard deviation) of				Spermatogenesis (% of tubules with mature sperm)
Age period (weeks)	Daily dose (mg/male)	Body wt. (g)	Testes (mg)	Spleen (mg)	Thymus (mg)	
0–4 (6 males)	0.2 from day 1–20 2.0 from day 21–30	8.7 ± 1.0[a]	18.5 ± 8.3[a]	36.1 ± 3.7[a]	8.8 ± 3.3[a]	none retardation, picture resembles that in 2-week-old normal males
4–10 (15 males)	0.1	20.3 ± 0.9[a]	169.5 ± 13.3	89.3 ± 12.2	9.8 ± 3.3[a]	16.9[a]
	0.5	16.8 ± 1.3[a]	113.4 ± 13.4[a]	77.1 ± 12.1	3.5 ± 2.5[a]	2.6[a]
	1.0	16.0 ± 1.4[a]	127.7 ± 21.3[a]	73.1 ± 11.8	not detectable	18.3[a]
8–14 (19 males)	0.1	22.7 ± 2.2	132.9 ± 28.3[a]	165.9 ± 53.7	11.3 ± 3.8[a]	6.8[a]
	0.5	20.8 ± 1.1	149.5 ± 5.4	114.1 ± 34.4	2.8 ± 0.5[a]	13.9[a]
	1.0	19.6 ± 0.9	131.7 ± 18.4[a]	112.0 ± 18.8	3.8 ± 0.3[a]	9.1[a]
	a single dose					
4 (18 males)	0.1	14.9 ± 0.9	n.d.		52.0 ± 17.2[a]	n.d.
	1.0	14.0 ± 1.0			28.9 ± 6.0[a]	
4 (8 males)	–	14.4 ± 0.9	99.2 ± 11.5	105.4 ± 27.8	70.5 ± 6.8	–
10 (5 males)	–	24.2 ± 0.8	185.1 ± 23.5	82.8 ± 6.9	48.8 ± 4.1	38.9
14 (8 males)	–	23.0 ± 2.3	163.2 ± 16.8	121.9 ± 2.3	49.3 ± 14.9	47.3

[a] Values differ significantly at the 0.05 level (by the *t*-test) from the appropriate control.

cortex. Disintegration of nuclei and the presence of their phagocytized remnants in reticulocytes was occasionally observed in the medulla (Figure).

The effects of steroid hormones on lymphoid tissues and through this on immunity were demonstrated in various experimental situations[8–13]. Following the long-term administration of CA, the changes in the weight and microstructure of the thymus and spleen essentially resembled those after repeated doses of testosterone[11]. The picture seen 48 h after a single dose of CA is reminiscent of the effect of cortisone[14], except for the signs of cell disintegration being less frequent with CA inspite of the striking depletion of lymphocytes. This difference might indicate that the acute lympholytic effect of cortisone[15] is not produced by CA which may rather accelerate the migration of lymphocytes from the thymus, as does testosterone when administered repeatedly[11].



Thymus of a 30-day-old male mouse 48 h following a single dose of 1 mg CA: the normal microscopic structure reversed, cortical lymphocytes depleted. Fixative neutral formol, stained with haematoxylin-eosin. ×250.

These side-effects of CA on the lymphoid system should be taken into account when the drug is considered for a therapeutic treatment in clinical medicine. Such drastic alterations of the lymphoid system as observed in our experiments can reasonably be expected to weaken the immune defense capacity of which the undesirable immediate consequences are obvious; this capacity is, however, also hoped to serve a positive function in the 'immune surveillance' of neoplasms, particularly in trapping the newly arizing tumour cells of which the frequency is hard to estimate as long as the immune system is functioning normally[16,17].

* This investigation received financial support from the World Health Organization.

[1] F. Neumann, R. von Bernswordt-Wallrabe, W. Elger, H. Steinbeck, J. D. Hahn and M. Kramer, Rec. Progr. Hormone Res. *26*, 337 (1970).
[2] F. Ott and H. Hoffet, Schweiz. med. Wschr. *93*, 1812 (1968).
[3] U. Laschet and L. Laschet, J. neuro-visceral Relations Suppl. *10*, 388 (1971).
[4] W. W. Scott and J. C. Wade, J. Urol. *101*, 81 (1969).
[5] W. W. Scott, Cancer *32*, 1119 (1973).
[6] M. Vojtíšková and M. Poláčková, Folia biol., Praha *19*, 381 (1973).
[7] M. Poláčková, V. Viklický and M. Vojtíšková, Folia biol., Praha *21*, 20 (1975).
[8] T. F. Dougherty, Physiol. Rev. *32*, 379 (1952).
[9] H. S. Kaplan, C. S. Nagareda and M. B. Brown, Rec. Progr. Hormone Res. *10*, 293 (1954).
[10] R. J. Graff, M. A. Lappé and G. D. Snell, Transplantation *7*, 105 (1969).
[11] M. Frey-Wettstein and C. G. Craddock, Blood *35*, 257 (1970).
[12] D. Eidinger and T. J. Garrett, J. exp. Med. *136*, 1098 (1972).
[13] M. Kotani, Y. Nawa and H. Fujii, Experientia *30*, 1343 (1974).
[14] H. Blomgren and B. Andersson, Cell. Immun. *1*, 545 (1971).
[15] M. H. Claesson and C. Røpke, Acta path. microbiol. scand. *76*, 378 (1969).
[16] D. Keast, Lancet *2*, 710 (1964).
[17] G. Klein, in *Immunological Aspects of Neoplasia* (Williams and Wilkins, Baltimore 1975), p. 21.