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SHORT COMMUNICATION

Initiation of enzyme-altered foci by the synthetic steroid cyproterone acetate in rat liver foci bioassay

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Cyproterone acetate (CPA) is a synthetic steroid which is used in oral contraceptive and anti-androgen formulations. It has previously been classified as a tumor promoter in rat liver. Recent studies have shown that CPA induces DNA repair synthesis in isolated hepatocytes, and this implies that CPA is genotoxic. We studied the initiating activity of CPA *in vivo* by means of a rat liver foci bioassay, using weanling female Sprague–Dawley rats. The results show that CPA induces adenosine-triphosphatase-deficient and γ-glutamyltranspeptidase-positive putative preneoplastic foci in a dose-dependent manner. This indicates that CPA has not only promoting but also initiating activity and may therefore act as a complete carcinogen in rat liver.

Cyproterone acetate (CPA*) is a synthetic steroid which is used in oral contraceptive and anti-androgen formulations. In a chronic carcinogenicity study CPA has been shown to increase the liver tumor rate in rats (1,2). The tumorigenicity of the steroid in rat has been attributed to a tumor-promoting activity of this compound (3). This assumption is confirmed by several findings. CPA stimulates liver growth in rat (4), and DNA synthesis and mitosis in primary cultures of rat hepatocytes (5). Moreover CPA induces a higher proliferative activity in preneoplastic cells of γ-glutamyltranspeptidase (GGT)-positive foci than in unaltered liver cells *in vivo* and *in vitro* (6,7).

Until recently there was no evidence that CPA had genotoxic or tumor-initiating activities. The steroid was not mutagenic in *Salmonella typhimurium* with and without metabolic activation (8), and did not induce GGT-positive liver cell foci when tested in a rat liver foci bioassay using the Solt–Farber protocol (9). However, we have recently shown that CPA induces DNA repair synthesis in isolated rat hepatocytes (7), which implies that CPA is genotoxic. In view of this finding an initiating potential of CPA *in vivo* should be taken into account. We therefore re-examined whether the synthetic steroid has any initiating activity using our protocol for the rat liver foci bioassay, which has been shown to be more sensitive than other test protocols (10).

The rat liver foci bioassay was performed as described earlier (11). Twenty-eight female Sprague–Dawley rats (inbred strain, Neuherberg), 22 days old, were used. They were kept as described previously (11). Food (Altromin, Lage) and drinking water were given *ad libitum*.

CPA was a kind gift of Schering AG, Berlin. It was suspended in olive oil immediately before use in concentrations of 12.5, 25 and 50 mg/ml. A concentration of 2 ml/kg body wt of the suspensions was given to the animals by gavage between 9.00 and 10.00 a.m. In order to test the initiating potential of the steroid, four groups of six animals each were treated with the vehicle only, and 25, 50 and 100 mg CPA/kg body wt, on five consecutive days. One week after the last application the animals received 10 mg of Clophen A50/kg in 2 ml of olive oil/kg body wt p.o. twice weekly for 11 consecutive weeks as tumor promoter. Clophen A50 is a technical mixture of polychlorinated biphenyls (PCBs) with a mean chlorine content of 50%. One group of four animals remained untreated.

The animals were killed under ether anesthesia by decapitation 4 days after the last treatment with PCBs. Cryostat sections from the median and left liver lobe were stained for adenosine-5′-triphosphatase (ATPase) and GGT. The number and area of ATPase-negative and GGT-positive foci with a size of at least three cells were measured by means of a semi-automatic picture analyzer (Videoplan, Kontron, Eching, Germany). The single sections (1 cm^2) were taken ~400 μm apart from each other. In total eight sections for ATPase, and four sections for GGT were evaluated, corresponding to a total area of 8 and 4 cm^2 respectively.

Treatment of rats with CPA and PCBs resulted in a slight (~4%) but significant reduction of the body weight compared to the untreated and vehicle/PCB-treated rats (Table I). However, this effect was not dose dependent. Liver weights and relative liver weights were increased in all groups receiving PCBs.

In the untreated animals, few ATPase-deficient and GGT-positive foci were observed (Table II). The repeated treatment with Clophen A50 (group 2) enhanced the number and area of foci compared to the untreated animals (group 1). This may be related to a weak initiating potential of the PCB mixture used, which may lead to a syncarcinogenic effect. Earlier observations, however, have shown that the number of foci observed after PCB treatment is not dose dependent (12). Therefore, the increase in the number of foci is most likely related to promotion of pre-existing spontaneously initiated cells (3,13). The number and area of ATPase-deficient and GGT-positive foci was significantly enhanced in the animals treated with 50 and 100 mg CPA/kg body wt compared to the PCB-treated controls. At the lower dose of 25 mg/kg body wt the number of ATPase-deficient foci was significantly higher than in the PCB-treated controls.

The question whether some synthetic estrogens and progestins have not only a tumor-promoting but also a tumor-initiating potential has previously been the matter of some controversy (14–16). Nearly 10 years ago Schuppler *et al.* (9) studied the tumor-initiating activity of CPA, ethinyl estradiol and other sex steroids in the liver using the initiation–selection protocol of Solt and Farber (17). In this study male rats were treated i.p. with a single dose of 100 mg CPA/kg body wt, 18 h after two-thirds hepatectomy. Since the synthetic steroids did not induce GGT-positive foci, the authors concluded that the compounds tested are not initiating carcinogens. In contrast, Ghia and Mereto (18) reported that ethinyl estradiol initiated GGT-positive foci in the livers of female rats when given in the diet at a concentration of 10 p.p.m. for 6 weeks. The total dose of the estrogen administered was lower than in the study of Schuppler *et al.* (9).

In the present study treatment with CPA significantly increased the number and the area of ATPase-deficient and GGT-positive

*Abbreviations: CPA, cyproterone acetate; GGT, γ-glutamyltranspeptidase; PCB, polychlorinated biphenyl; ATPase, adenosine-5′-triphosphatase.

Table I. Liver and body weight of female Sprague–Dawley rats treated with CPA and PCB mixture Clophen A50

Group	Treatment	Body weight (g)	Liver weight (g)	Liver wt/ body wt (%)
1	−/− (4)[a]	271.5 ± 2.4[b]	9.3 ± 0.6*	3.5 ± 0.2*
2	olive oil/PCB (6)	273.5 ± 7.5	10.9 ± 0.4	4.0 ± 0.1
3	5× 25 mg CPA/PCB (6)	260.2 ± 10.0*	10.3 ± 0.5	4.0 ± 0.3
4	5× 50 mg CPA/PCB (6)	262.2 ± 5.3*	11.1 ± 0.4	4.2 ± 0.1*
5	5× 100 mg CPA/PCB (6)	260.0 ± 3.3*	11.1 ± 0.5	4.3 ± 0.2

[a]Numbers of animals in parenthesis.
[b]Mean ± SD.
*Significantly different from group 2; Student's *t*-test, $P < 0.025$.

Table II. Dose-dependent incidence of enzyme-altered foci in livers of female Sprague–Dawley rats treated with CPA and PCB mixture Clophen A50

		ATPase-deficient foci		GGT positive foci	
Group	Treatment	Number (no./cm^2)	Area (mm^2/cm^2)	Number (no./cm^2)	Area (mm^2/cm^2)
1	−/− (4)[a]	0.02 ± 0.05[b]	0.005 ± 0.004	0.6 ± 0.5	0.005 ± 0.004
2	olive oil/PCB (6)	1.6 ± 0.5*	0.027 ± 0.016	1.2 ± 0.6*	0.013 ± 0.0103
3	5× 25 mg CPA/PCB (6)	3.6 ± 0.9**	0.049 ± 0.011	1.7 ± 0.8	0.055 ± 0.052
4	5× 50 mg CPA/PCB (6)	5.1 ± 1.0**	0.098 ± 0.022**	3.9 ± 0.9**	0.123 ± 0.056**
5	5× 100 mg CPA/PCB (6)	9.6 ± 2.9**	0.122 ± 0.033**	6.7 ± 2.4**	0.150 ± 0.059**

[a]Numbers of animals in parenthesis.
[b]Mean ± SD.
*Significantly different from group 1, *t*-test, $P < 0.025$.
**Significantly different from group 2, *t*-test, $P < 0.001$.

foci compared to treatment with PCBs only. The dose-dependent induction of preneoplastic foci by CPA indicates an initiating potential of the steroid. The discrepancy between the results of the study of Schuppler *et al.* (9) and those of the present investigation may be due to several reasons.

CPA was administered five times in the present study, whereas a single dose was given in the earlier study. The initiation–promotion protocol for the rat liver foci bioassay using weanling female Sprague–Dawley rats and Clophen A50 as tumor promotor has proven to be more sensitive than the protocol of Tatematsu *et al.* (19), with respect to the number of foci induced by genotoxic carcinogens (10). The protocol according to Tatematsu *et al.* (19) represents a modification of the Solt–Farber system that was used by Schuppler *et al.* (9). In both protocols, adult male rats are used. The use of female rats in the present study appears to be especially important, since the tumorigenic activity of CPA has been shown to be higher in female rats compared to males (1,2). In contrast, another 2 year tumorigenicity study did not confirm these results, since proliferative changes in the liver were more frequent in males than in females (P.Günzel, personal communication).

Recent results indicate that the genotoxic activity of CPA differs significantly in the two genders. The level of hepatic DNA adducts induced by CPA treatment in rats is at least 50-fold higher in females compared to males, and the induction of DNA repair synthesis could be detected in female rats only (20).

The present study shows that CPA has not only promoting but also initiating activity in rat liver. This finding is in accordance with other observations indicating genotoxic activity of CPA *in vitro* and tumorigenicity *in vivo*. Taken together, the results indicate that CPA acts as a complete carcinogen in *female* rats.

References

1. Schuppler,J. and Günzel,P. (1976) Cyproterone acetate. *Lancet*, **ii**, 688.
2. Schuppler,J. and Günzel,P. (1979) Liver tumors and steroid hormones in rats and mice. *Arch. Toxicol., Suppl.*, **2**, 181–195.
3. Schulte-Hermann,R., Timmermann-Trosiener,I. and Schuppler,J. (1983) Promotion of spontaneous preneoplastic cells in rat liver as a possible explanation of tumor production by nonmutagenic compounds. *Cancer Res.*, **43**, 839–844.
4. Schulte-Hermann,R., Hoffman,V., Parzefall,W., Kallenbach,M., Gerhardt,A. and Schuppler,J. (1980) Adaptive responses of rat liver to the gestagen and anti-androgen cyproterone acetate and other inducers. II. Induction of growth. *Chem.-Biol. Interactions*, **31**, 287–300.
5. Parzefall,W., Monschau,P. and Schulte-Hermann,R. (1989) Induction by cyproterone acetate of DNA synthesis and mitosis in primary cultures of adult rat hepatocytes in serum free medium. *Arch. Toxicol.*, **63**, 456–461.
6. Schulte-Hermann,R., Ohde,G., Schuppler,J. and Timmermann-Trosiener,I. (1981) Enhanced proliferation of putative preneoplastic cells in rat liver following treatment with the tumor promoters phenobarbital, hexachlorocyclohexane, steroid compounds, and nafenopin. *Cancer Res.*, **41**, 2556–2562.
7. Neumann,I., Thierau,D., Andrae,U., Greim,H. and Schwarz,L.R. (1992) Cyproterone acetate induces DNA damage in cultured rat hepatocytes and preferentially stimulates DNA synthesis in γ-glutamyltranspeptidase-positive cells. *Carcinogenesis*, **13**, 373–378.
8. Lang,R. and Redmann,U. (1979) Non-mutagenicity of some sex hormones in the Ames *Salmonella*/microsome mutagenicity test. *Mutat. Res.*, **67**, 361–365.
9. Schuppler,J., Dammé,J. and Schulte-Hermann,R. (1983) Assay of some endogenous and synthetic sex steroids for tumor-initiating activity in rat liver using the Solt–Farber system. *Carcinogenesis*, **4**, 239–241.
10. Oesterle,D., Gerbracht,U., Deml,E., Schlatterer,B. and Eigenbrodt,E. (1989) Comparison of three rat liver foci bioassays—incidence of preneoplastic foci initiated by diethylnitrosamine. *Carcinogenesis*, **10**, 1891–1895.
11. Oesterle,D. and Deml,E. (1983) Promoting effect of polychlorinated biphenyls on development of enzyme-altered islands in livers of weanling and adult rats. *J. Cancer Res. Clin. Oncol.*, **105**, 141–147.
12. Oesterle,D. and Deml,E. (1984) Dose-dependent promoting effect of

polychlorinated biphenyls on enzyme-altered islands in livers of adult and weanling rats. *Carcinogenesis*, **5**, 351–355.
13. Boutwell,R.K. and Sivak,A. (1974) The function and mechanism of promoters of carcinogenesis. *Crit. Rev. Toxicol.*, **2**, 419–443.
14. Porter,L.E., van Thiel,D.H. and Eagon,P.K. (1987) Estrogens and progestins as tumor inducers. *Semin. Liver Dis.*, **7**, 24–30.
15. Degen,G.H. and Metzler,M. (1987) Sex hormones and neoplasia: genotoxic effects in short term assays. *Arch. Toxicol.*, **10**, 264–278.
16. Sekeris,C.E. (1991) Hormonal steroids act as tumour promoters by modulating oncogene expression. *J. Cancer Res. Clin. Oncol.*, **117**, 96–101.
17. Solt,D. and Farber,E. (1976) New principle for analysis of chemical carcinogens. *Nature*, **283**, 701–703.
18. Ghia,M. and Mereto,E. (1989) Induction and promotion of γ-glutamyltranspeptidase-positive foci in the liver of female rats treated with ethinyl estradiol, clomiphene, tamoxifen and their associations. *Cancer Lett.*, **46**, 195–202.
19. Tatematsu,M., Hasegawa,R., Imaida,K., Tsuda,H. and Ito,N. (1983) Survey of various chemicals for initiating and promoting activities in a short-term *in vivo* system based on generation of hyperplastic nodules in rats. *Carcinogenesis*, **4**, 381–386.
20. Topinka,J, Andrae,U., Schwarz,L.R. and Wolff,T. (1993) Cyproterone acetate generates DNA adducts in rat liver and in primary rat hepatocyte cultures. *Carcinogenesis*, **14**, in press.

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