Carcinogenicity of cyproterone acetate in the mouse

M.J. Tucker, A.E. Kalinowski and T.C. Orton

Safety of Medicines Department, ZENEGA Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

To whom correspondence should be addressed

The synthetic progestin cyproterone acetate (CPA) has been shown to be a hepatocarcinogen in the rat, but little is known of its effects in mice. A 52 week CPA study in the mouse strain C57Bl/10J has been reported not to produce liver tumours, although CPA induced significant liver enlargement and induction of the mixed function oxidase CYP3A. The present study is a further investigation of the effects of CPA in mice of the C57Bl/10J strain dosed with CPA, compared with none in the controls (the strain has a consistently low level of spontaneous liver tumours (1)). We considered that the lack of hepatic tumours after 12 months treatment in C57Bl/10J mice might reflect a temporal difference in response, rather than a resistance to tumour development, and this investigation reports the effects of 104 weeks treatment.

Materials and methods

Animals and dosing

Tablets (50 mg) of Androcur™ (Schering AG, UK) were ground to a fine powder using a cyclotec 1093 sample mill (1 mm screen) and incorporated into powdered irradiated R & M No. 1 (modified) diet (Special Diet Services, UK) at a level of 800 p.p.m. Seven-week-old C57Bl/10J mice, weight range 23-31 g for males and 18-22 g for females, were supplied by the Animal Breeding Unit at ZENEGA Pharmaceuticals (Alderley Park, UK). The animals had a 10 day acclimatization period before dosing commenced, during which they were monitored for signs of illness. They were housed four/cage (controls) or five/cage (CPA-dosed) in solid plastic cages in ventilated cabinets; environmental conditions included a 12 h light/dark cycle, ambient room temperature of 21 ± 2°C and humidity of 50 ± 15%. Water and diet were available ad libitum. The animals were randomly divided into a control group of eight/sex fed the unmedicated diet and a group of 40/sex fed 800 p.p.m. CPA for 104 weeks. This dose had been selected as the maximum tolerated dose with respect to the liver based on the results of previous studies, but the diet intake was not recorded so the dose received was unknown. All animal work was performed in accordance with the principles of the United Kingdom Animal (Scientific Procedures) Act 1986. All but four animals (which were cannibalized) were necropsied and the livers weighed prior to fixation. All major organs were sampled, processed by standard techniques and examined histologically.

Immunohistochemistry

For assessment of different cell types, sections of pituitary and pancreas from three control and 11 CPA mice were processed for follicular stimulating hormone (pituitary), luteinizing hormone (pituitary), adrenocortictropic hormone (pituitary), prolactin (pituitary), insulin (pancreas), glucagon (pancreas) and somatostatin (pancreas) using the peroxidase-antiperoxidase method. Sections were incubated with antisera to follicular stimulating hormone (rabbit anti-human; Dako) at a dilution of 1 in 20, luteinizing hormone (rabbit anti-human; Dako) at a dilution of 1 in 500, adrenocorticotrophic hormone (rabbit anti-human; Dako) at a dilution of

Introduction

Cyproterone acetate (6-chloro-17-acetoxy-1,2-methylene-pregna-4,6-diene-3,20-dione; CPA*) is a synthetic steroid widely used in humans: it is a potent progestin, derived from hydroxy-progesterone, with anti-androgenic activity and it is used in contraceptive drugs and in the treatment of prostate cancer and other androgen-dependent conditions (1). In the rat, CPA increased the incidence of tumours in the liver after long-term feeding of high doses (2,3), with a higher incidence in females. Further investigations suggested that this tumorigenic effect was via epigenetic mechanisms, due to promoting activity similar to that of phenobarbital and other enzyme-inducing xenobiotics (4-6). This was supported by the lack of activity in standard tests for genotoxicity, such as the Ames' Salmonella test (7) and the Solt–Farber test for preneoplastic foci in the rat liver (8). Recent work, however, has shown that CPA has genotoxic properties (DNA adduct formation) in rat liver and in rat hepatocyte cultures (9), as do the structural analogues of CPA, chlormadinone acetate and mestregest acetate, although the analogues show a lower level of genotoxicity than CPA (10). The effects of CPA in mice have not been extensively studied, but one long-term study with the analogue CMA did not produce liver tumours in mice or rats at a dose stated to be 200-400 times the human contraceptive dose of 2 mg (11). Doses of CPA vary with the clinical condition being treated; in prostate cancer doses as high as 250 mg/day have been used (1). At high doses (900 p.p.m. in diet) CPA has been shown to produce tumours in the livers of 40% of male CD1 mice after 12 months treatment, but not in the inbred C57Bl/10J, CD-1/Alpk mouse (short form C57Bl/10J), which is used in our laboratories for all oncogenicity studies (12). This latter strain has a consistently low level of spontaneous liver tumours ranging between 0 and 5% (13). We considered that the lack of hepatic tumours after 12 months treatment in C57Bl/10J mice might reflect a temporal difference in response, rather than a resistance to tumour development, and this investigation reports the effects of 104 weeks treatment.

Abbreviations: CPA, cyproterone acetate; PCNA, proliferating cell nuclear antigen; ACTH, adrenocorticotropic hormone.

© Oxford University Press

1473
Cumulative mortality
(number of animals)

Control - male n=8
800 ppm CPA - male n=40
Control - female n=8
800 ppm CPA - female n=40

8 16 24 32 40 48 56 64 72 80 88 96 104
Duration (weeks)

Fig. 1. Cumulative mortality in C57Bl/10J mice fed 800 ppm CPA in the diet for 104 weeks compared with controls fed the unmedicated diet.

Relative liver weight
(liver / body weight)

Control - male
800 ppm CPA - male
Control - female
800 ppm CPA - female

0 20 40 60 80 100
Animal Identity

Fig. 2. Individual relative liver weights (liver as a percentage of body weight) of mice fed 800 ppm CPA in the diet for 104 weeks compared with controls fed the unmedicated diet.

M.J. Tucker, A.E. Kalimowski and T.C. Orton

Results

Mortality in the CPA-dosed mice remained low for the first 40 weeks of the study, but thereafter increased rapidly (Figure 1). All females had died by week 97 and only four males survived to the end of the 104 week dosing period. The chief cause of death in females was uterine enlargement, and in males neoplastic diseases. Liver weights of treated and control mice could not be compared statistically because of individual differences in duration of treatment and the small number of controls, but Figure 2 shows the individual liver weights relative to body weight and demonstrates the large increase in liver weight in CPA-dosed mice, which, in males, was in excess of 100% compared with controls.

The incidence of liver tumours (Table 1) shows that 44% of males and 22% of females dosed with CPA developed liver tumours (two/sex had both a benign and a malignant liver tumour). There were no liver tumours in the controls, a finding consistent with the continuing low incidence in the strain and the small control group size. The tumours in the CPA-dosed mice were generally small, the first being an incidental finding in an animal which died at 65 weeks. One male which survived to the end of the 104 weeks of dosing did not have a liver tumour. The sex difference in incidence may not be real, as the tumours were a late development, with 7/19 tumours in males occurring after all the females had died. The malignant tumours were poorly differentiated (Figure 3), but metastases were not identified. PCNA staining showed an increase in proliferating cells, when compared with controls, particularly in the tumours, but also in the non-tumour bearing liver of CPA-dosed mice (data not shown). The livers showed marked hepatocyte hypertrophy, increased fat and glycogen (Figure 4) and hepatocellular necrosis of single cells or small foci.

Eighty six per cent of males and 97% of females dosed with CPA developed single, adenomatous polyps of the gastric pyloric antrum (Figure 5). Several control animals were also reported to have nodules/masses in the stomach at necropsy, although these lesions were not similar in location or appearance to those in the CPA-dosed animals; histological examination of the stomachs of control mice only identified some minimal inflammation in a few. This accords with the low spontaneous incidence of gastric tumours in the strain, which has not exceeded 1% in the control animals of any study at
Fig. 4. Liver from male dosed with 800 p.p.m. CPA showing macrovesicular hepatocyte fat vacuolation. H&E. ×128.

Fig. 5. Single well-differentiated adenomatous polyp of the gastric pyloric antrum. H&E. ×8.

Fig. 6. Islet cell hyperplasia of pancreas in (a) male CPA-dosed compared with (b) male control. H&E. ×8. I indicates pancreatic islets.

Zeneca. The polyps in the CPA-dosed mice probably did not contribute to death, except possibly in the case of a few animals which survived 90 weeks of dosing, where superficial ulceration may have been a contributing factor. Histologically, the polyps have a typical benign appearance; they were well differentiated with only a few showing dysplasia and downgrowth into the stalk. The incidence of several other tumour types were high compared with our historical database, but the small control group precludes statistical evaluation, e.g. a 10% incidence of myeloid leukaemia in CPA mice compared with a zero incidence in controls and <1% in any study in the Zeneca database. Conversely, composite lymphomas, the most common tumour in the strain (13), were reduced in incidence, occurring in 7/8 male and 3/8 female controls, compared with 1/39 and 1/37 CPA-dosed animals.

The most important non-neoplastic change induced by CPA was hyperplasia of the pancreatic islets (Figure 6), which was found in all males and 88% of females, the severity being more marked in females. Not all of the islets in one pancreas appeared enlarged and most retained their normal histological appearance; immunostaining demonstrated that they had a normal ratio of cells, the majority being insulin-secreting cells. Although occasional mitotic figures were seen in the islets, PCNA staining did not show any significant increase in cell proliferation.

Other effects were associated with the known hormonal activity of CPA and included atrophy of the seminal vesicles, uterine endometrial hyperplasia with multiple decidual reac-
tions, and adrenocortical atrophy. Immunostaining of the pituitary glands did not reveal any changes in cytology.

Discussion

The hepatocarcinogenic effect in this study supports the observation in CD1 mice (12) and confirms the contention that CPA is carcinogenic in the liver of C57BI/10J mice, but that the latent period for induction is longer than in CD1 mice. Unlike the rat, where the female has been shown to be more susceptible (23), no such conclusion could be drawn for the mouse because of premature deaths in the females. In the rat the hepatocarcinogenic effect of CPA has been related to the formation of DNA adducts (9) and the promotion of these initiated hepatocytes via mitogenic activity and reduced apoptosis (6, 14, 24). Although the formation of DNA adducts has not been examined in the mouse liver, the enlargement of the liver seen in this study, and the increase in cell proliferation seen in this and other studies in mice (12, 15), suggest that the hepatocarcinogenic effect in mice is via a similar mechanism.

The mechanism for induction of gastric polyps is not known. Spontaneous polyps are rare in mice, although they have been recorded in a few strains, including C57BI (16, 17), but there are no reports of induction by hormonally active compounds. Progesterone receptors have been located in gastric tumours and to a lesser extent in normal gastric tissue (19) and it is possible that the effects of CPA in the stomach are mediated through effects on these receptors.

Islet cell hyperplasia in the rat pancreas has been reported with a luteinizing hormone releasing hormone agonist (20) and attributed to effects on pituitary growth hormone secretion. Hyperplasia has also been seen with the corticosteroid Deflazacort™ (21) in the rat and, as with CPA, the hyperplastic islets were composed chiefly of insulin-secreting cells, although there were no changes in blood sugar or plasma insulin levels. In the CPA-dosed mice, the changes in liver glycogen and the adrenocortical atrophy indicate that CPA has glucocorticoid activity; it has been shown that CPA produces adrenocortical atrophy in the rat by feedback inhibition of pituitary adrenocorticotropic hormone (ACTH) secretion (22). Although immunostaining of the pituitary in this study did not indicate any effects on the number of ACTH cells present, the atrophy of the adrenal cortex is probably indicative of suppression of ACTH secretion. It may be that the islet cell hyperplasia is secondary to the adrenal atrophy and glucocorticoid activity. The effects in stomach and pancreas were unexpected, and incidental, findings in this study and further work will be necessary to elucidate the mechanism of induction of these effects.

Acknowledgements

We thank Amanda Wright, John Dakins, and Steve Moore for technical assistance.

References

20. US Food and Drug Administration. Advisory Committee Meeting Minutes. 20th meeting, 26th April.

Revised on December 21, 1995, revised on April 1, 1996, accepted on April 12, 1996.