Follow-Up Study of Children with Precocious Puberty Treated with Cyproterone Acetate

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A total of 1840 children and adolescents treated with cyproterone acetate (CPA) to block gonadal function, as a treatment for precocious puberty, short stature and other disorders, were registered to survey for the risk of developing hepatic tumors. Patients responding to follow-up numbered 1552 (85%). The cumulative dose and duration of CPA therapy for boys and girls were 110.4g and 2.6 years, and 122.9 g and 2.8 years, respectively. Among the 1552 patients, five hepatoma cases were found. Four underwent successful surgery and remain alive and well to date. Two of the 5 cases had been given more than 500g, the other 3 more than 1000 g, of CPA. Three had also been given androgens before CPA administration. Although further follow-up is necessary to monitor for the development of adenoma and hepatoma, the risk of developing these tumors among patients to whom limited doses of CPA were administered appears to be negligible. J Epidemiol, 1997; 7: 173-178.

cyproterone acetate, precocious puberty, liver tumor, adverse effects

Cyproterone acetate (CPA; 6-chloro-17-alpha-acetoxy-1,2 alpha-methylenepregna-4,6-diene-3,20-dione) is a synthetic progestagen that suppresses gonadotropin secretion and blocks androgen action as a competitive inhibitor of androgen receptors. Since its introduction, CPA has been used in the treatment of male hypersexuality, deviationism, and prostatic carcinoma. It is also used for treatment of women with androgen-related diseases, and small doses have been widely used as oral contraceptives. In Japan, CPA was introduced in 1983 to treat precocious puberty, because of its gonadal suppressive action resulting in better growth. CPA was subsequently used to treat growth hormone (GH) deficient children and non-GH deficient short stature. Three cases of hepatocellular carcinoma have been reported, however, in CPA patients in Japan. To date, 6 cases of hepatocellular carcinoma have also been reported in the international literature. The former appeared to be fortuitous, and the latter risk was lower than that associated with other contraceptives (personal communication; Multicentric International Liver Tumor Study: MILTS).

In Japan, it was found to be important to monitor children and adolescent CPA users for liver tumors, because idiopathic precocious puberty is no longer treated with CPA in other countries worldwide. Thus, it is worthwhile to ascertain the situation in Japan. It was quite routine to delay puberty for a few years, in the early 60s and 70s, before LHRH superagonists became available.

The mortality rate from primary liver cancer in Japan is less than 0.2 per 100,000 for people under 20 years old. Thus, three cases of hepatoma among CPA users suggest a high risk asso-
associated with CPA treatment\textsuperscript{10}. An ad hoc committee was established to register all patients who used CPA and to follow-up these patients focusing on the development of hepatic abnormalities including hepatic adenoma or hepatoma.

**PATIENTS AND METHODS**

From 1993 to the end of 1996, 36 main hospitals and clinics in Japan were included in the survey (see annex). It was estimated that more than 70\% of all patients receiving CPA therapy were managed by these hospitals, according to drug company sales records. Pediatricians in charge completed the questionnaire for each patient receiving CPA in their hospitals. The contents of the questionnaire included the diagnosis, details of CPA therapy, such as the start and the final date of the prescription, duration of therapy, compliance, dosage, and other drugs used simultaneously. Results from ultrasonography, liver function tests, and serum alpha-fetoprotein measurement were also recorded by the physicians in charge, if these tests had been carried out. Registered patients were requested to receive follow-up health check-ups to detect possible liver tumors by either ultrasonography or alpha-feto protein. Physicians in charge were consulted if there were any problems concerning the data. No control group was included in this surveillance study, i.e. no patients with precocious puberty but who did not receive CPA treatment were followed. The data management and analysis were done with Excel, SAS, and SPSS.

**RESULTS**

In all, 1,552 questionnaires were returned, for a response rate of 85\%. Among these cases, 582 were males and 970 were females. The basic diseases included idiopathic precocious puberty and several varieties of short stature with children entering puberty who were still small, such as growth hormone deficiency (GHD), and non-GHD short stature (Table 1). Among males, GHD and non-GHD short stature were the most common diseases. The other rare diseases were congenital adrenal hyperplasia with central precocious puberty, organic precocious puberty and testo-toxicosis. Among the females, there were 324 cases of idiopathic precocious puberty. In addition to the 177 cases of non-GHD short stature in females, there were 54 of congenital adrenal hyperplasia, 37 of organic precocious puberty, 24 of McCune-Albright syndrome, and 120 of other miscellaneous causes.

On average, males began to take CPA at around 12.1 years old, while females started therapy earlier (9.8 years). At the end of the survey, 276 of the 1552 cases were still taking CPA. The mean duration of CPA use was 2.6 years in males, and 2.8 years in females. 33.1\% used more than 3 years, 20.4\% used more than 4 years, and 11.9\% used more than 5 years. Daily doses of CPA were 50, 100, 150, or 200 mg per day according to body weight. The lowest dose was 50 mg a week, and the highest dose was 600 mg per day. High doses were given in the 1980s to cases who did not respond well to CPA. The total amounts of CPA administered were 110.4 g (median 76.2g, range 0.3-737.5 g) and 122.9 g (median 84.7g, 0.8-1218.0 g) for male and female patients, respectively. More than 200 g had been given to 17\% patients, and more than 250 g in 10.2\% patients. More than 500 g CPA had been given to 24 patients.

Many other drugs were also prescribed, and growth hormone was the most common combination drug (642 cases). The other frequently used drugs were LH-RH analog (304 cases, glucocorticoid (144 cases), thyroxin (142 cases), progesterin (107 cases), mineral corticoid (66 cases), and androgenic steroids, such as stanozolol (52 cases).

By the end of 1995, there were 43 cases who had abnormal ultrasonographic results, 56\% of whom were found to have a

| Disease category                     | Male | %    | Female | %    | Total | %    |
|--------------------------------------|------|------|--------|------|-------|------|
| Idiopathic precocious puberty        | 37   | 6.4  | 324    | 33.4 | 361   | 23.3 |
| Organic precocious puberty           | 27   | 4.6  | 37     | 3.8  | 64    | 4.1  |
| Congenital adrenal hyperplasia       | 37   | 6.4  | 54     | 5.6  | 91    | 5.9  |
| McCune Albright syndrome             | 0    | 0.0  | 24     | 2.5  | 24    | 1.6  |
| Testotoxicosis                       | 3    | 0.5  | 0      | 0.0  | 3     | 0.2  |
| Short stature with early puberty     |      |      |        |      |       |      |
| Growth hormone deficiency (GHD)      | 296  | 50.9 | 249    | 25.7 | 545   | 35.0 |
| Turner syndrome                      | 0    | 0.0  | 18     | 1.9  | 18    | 1.2  |
| Non-GHD short stature                | 132  | 22.7 | 177    | 18.3 | 309   | 19.9 |
| Subclassification unknown            | 4    | 0.7  | 3      | 0.3  | 7     | 0.5  |
| Other diseases                       | 41   | 7.0  | 83     | 8.6  | 124   | 8.0  |
| Unclear                              | 5    | 0.9  | 1      | 0.1  | 6     | 0.4  |
| Total                                | 582  |      | 970    |      | 1552  |      |
fatty liver. Twenty patients had abnormal results on liver function tests, including GPT, GOT, gamma-GTP and choline esterase, and 5 cases had levels higher than the normal range of alpha-fetoprotein. Two had returned to normal in the second follow-up survey. The maximum dosage and the total amount of CPA for those with abnormal findings were higher than for those with normal findings (194.1 mg/day versus 164.8 mg/day, 198.0 g versus 112.3 g, respectively) (p<0.01). The duration of treatment was slightly longer in these patients but this was not statistically significant (3.1 years versus 2.6 years).

Follow-up status of the patients is shown in Table 2. There were 7 deaths recorded in the follow-up survey (Table 3). One died of liver cancer, 1 in a traffic accident, 2 of cerebral hemorrhage, 1 after a cardiac operation (the patient suffered from both Turner's syndrome and coarctation of the aorta), 1 of a brain tumor, and 1 due to an epileptic attack.

Table 2 shows the characteristics of the five heptoma cases. The first 3 were reported previously. Briefly, the first case started CPA treatment when she was 13 years old, with a diagnosis of Russell-Silver syndrome. Prior to CPA usage, stanozolol (1mg/day) had been administered for 10 months. CPA was taken for 3 years at an initial dose of 100 mg per day, subsequently increased to 200-250 mg/day to stop menstruation. About 7 years after the cessation of CPA treatment, a tumor mass was found by ultrasonography in the right hepatic lobe. The level of LDH, alkaline phosphatase, gamma-glutamyl transferase (gamma-GT), and alpha-fetoprotein in the blood were higher than normal. The tumor was resected, and diagnosed as a hepatocellular carcinoma. Two years later she died of respiratory insufficiency due to pulmonary metastasis at 23 years of age. The second and third female cases had Turner syndrome. CPA was given to the second girl at 200-300 mg/day when she was 10 years old. Stanozolol (0.5 mg/day) was also used for 7 years and 7 months. After treatment for 9 years, liver cancer was detected by computed tomography. The tumor was successfully resected and the elevated alpha-fetoprotein returned to a normal value. In the third girl, CPA (200 mg/day) was started at 9 years of age, and used for 7 years and 10 months. The patient was referred to the hospital due to a liver tumor when she was 19 years old, and a large hepatic tumor was seen on both ultrasonography and computed tomography. The alpha-fetoprotein level was high on admission. A right extended lobectomy was done. At the time of discharge, alpha-fetoprotein was still slightly higher than normal (290.1 ng/ml), but she was otherwise in good health. Both cases had also received recombinant growth hormone and LH-RH analogue (Buserelin) in addition to CPA.

The fourth case was a girl with McCune-Albright syndrome.

**Table 2. Follow-up status.**

| Follow-up                  | Frequency | %   |
|----------------------------|-----------|-----|
| Followed at out-patient clinic | 892       | 57.5 |
| Treatment at another hospital | 53        | 3.5  |
| Treatment completed         | 450       | 29.0 |
| Lost to follow-up           | 31        | 2.0  |
| Deceased                    | 7         | 0.5  |
| Total                       | 1,552     |      |

*Among whom 40 patients, who had been given more than 100 g of CPA, were confirmed to be alive through the permanent registration system for residents.

**Table 3. Deceased cases among CPA users.**

| Sex | Diagnosis                                      | Cause of death | Age of death | Age at onset of therapy | Duration | CPA dose |
|-----|------------------------------------------------|----------------|--------------|-------------------------|----------|----------|
| Case 1. | M Organic PP (brain tumor) | Brain tumor | 16y | 16y | 3m | 100mg/day, total 9g |
| Case 2. | M Noonan synd. Moyamoya-disease. | Post-operative | 21y | 12y | 2y4m | 150-200mg/day, total 115.5g |
| Case 3. | M GHD | Cerebral hemorrhage | 13y | 12y | 6m | 213.7g |
| Case 4. | F non-GHD short stature epilepsy, achondroplasia | Traffic accident | 17y | 12y | 5y7m | 36g |
| Case 5. | F Turner, coarctation aorta | Epilepsy | 13y | 12y | 6m | 36g |
| Case 6. | F GHD | Subarachnoidal hemorrhage | 14y | 13y | 2y | 100-300mg/day, total 60g |
| Case 7. | F Russell-Silver synd. | Hepatoma | 23y | 13y | 3y | 150-350mg/day, total 525.0g |

PP, precocious puberty; synd., syndrome.
Table 4. Hepatoma Cases of among CPA users.

| Case | Age (yr) | Sex | Diagnosis                      | Start dose (mg/day) | Dose (mg/day) | Period (yr) | Total dose (mg) | Interval (yr) | Other drugs       | Outcome |
|------|----------|-----|--------------------------------|---------------------|---------------|-------------|----------------|----------------|-------------------|---------|
| Case 1 | 22 F | Russell-Silver synd. | | 13 | 150-350 | 3 | 525g | 7 | Buserelin | deceased |
| Case 2 | 19 F | Turner synd. | | 10 | 200-300 | 9 | 560g | on use | rGh, Buserelin | alive |
| Case 3 | 19 F | Turner synd. | | 9 | 200 | 7y10m | 1100g | 2y4m | rGh, Buserelin | alive |
| Case 4 | 12 F | McCune-Albright synd. | | 4 | 200-300 | 4y+ | 988g | on use | Alfacalciferol | alive |
| Case 5 | 21 M | Adrenal hyperplasia | | 7 | 100-300 | 15y | 1562g | on use | Cortisol | alive |

rGh, recombinant growth hormone.

CPA was given at 200-300 mg/day for more than 4 years, starting at age 4 years. Somatostatin (100-150 ug/day) was also administered for one year. Bromocriptine (2.5-7.5 mg/day) was given to prevent gigantism. LH-RH analog (960-1290 ug/day) and alfacalciferol (D3) were also administered for 2 years. A hepatic tumor was found by ultrasonography, and a right hepatic lobectomy was performed. The fifth case was diagnosed as having simple virilizing type congenital adrenal hyperplasia at 3 years and 9 months of age. The patient had taken CPA (100-350 mg/day) since he was 6.5 years old. The total amount of CPA taken during the period of 7 years reached 1562g. In 1995, when he was 21 years old, a hepatic tumor was found by ultrasonography, and the level of alpha-fetoprotein at that time was more than 15,000 ng/dl. Left hepatic lobectomy was performed, and the alpha-fetoprotein level normalized. All cases had hepatocellular carcinoma (Edmondson I-II) with some adenomatous features. Neither antigens nor antibodies to hepatitis B or C virus were detected. Serum levels of alkaline phosphatase, LDH and gamma-GTP were found to be higher than normal before the liver tumors were diagnosed.

**DISCUSSION**

Formerly, CPA was used as the main treatment for precocious puberty, in Japanese children and adolescents, with the aim of stimulating growth via gonadal suppression. This study provides surveillance data focusing on the adverse effects of CPA therapy. Our data reveal that patients with hepatoma were given cumulative CPA doses of more than 500 g for over 2.5 years. Despite different underlying genetic conditions and different therapeutic regimens, including androgenic steroids, one common feature was long-term and high dose CPA treatment.

CPA treatment is only one of the common features in this surveillance. In four of the five cases, hepatomas were detected around 20 years of age, such that a weak promoting effect of CPA is a possibility.

Experimental studies have suggested that CPA has both promoting and initiating activity in the rat liver13-15. The genotoxic effects in the formation of hepatic tumors with long-term exposure in humans is assumed, because cultured human hepatic cells exposed to CPA in vitro showed altered DNA repair mechanism16. The CPA-DNA adducts persisted for several months, and some metabolites such as 3-gamma-OH-CPA or 3-OAc-CPA bound covalently to DNA in vitro, and 3-gamma-OH-CPA also bound DNA in vivo17,18. CPA strongly elicited DNA repair processes in primary cultures of hepatocytes from female rats18. On the contrary, CPA itself did not cause mutagenicity in rat liver cells19. Pharmacologically, CPA is a synthetic steroid which has both anti-androgenic and progestagenic effects. The complex interaction between CPA and sex hormone receptors may promote carcinogenesis20,21.

Although laboratory results have shown CPA to be genotoxic in cultured human hepatocytes, there is neither in vivo nor epidemiological evidence of this action to date, except for several case reports of hepatic carcinoma in patients given CPA therapy6,11. The current surveillance may provide further epidemiological clues as to the carcinogenic effect of CPA in humans with on-going follow-up.

Presently, the use of CPA as a component of oral contraceptives is restricted to only 2 mg per day. Patients with a history of having taken more than 500g of CPA are recommended to undergo close follow-up by ultrasonography and liver function tests, including alpha-fetoprotein measurement, at least once a year.

**CONCLUSION**

The present data provide epidemiological clues as to the role of CPA in hepatocarcinogenesis among children and adolescents taking this drug. The incidences and mortality rates of hepatic tumors in this age category are too small, with the exception of hepatoblastoma, for the meaningful calculation of a standardized incidence ratio (SIR).

Five cases of hepatocarcinoma among patients taking CPA constitute an unusually high number and the possibility of an association with long-term treatment with extremely high doses of CPA, at a young age, cannot be excluded. In order to ascertain the epidemiological relationship between CPA use and the development of hepatic tumors, further analytical study is required which would allow conclusions on causality to be drawn20.
Hepatoma Among Cyproterone Autate Uses

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