Synthesis and Anti-tumor Activity of a Fluorinated Analog of Medroxyprogesterone Acetate (MPA), 9α-Fluoromedroxyprogesterone Acetate (FMPA)

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We synthesized 9α-fluoromedroxyprogesterone acetate (FMPA) in order to test whether it is a more potent anti-angiogenic agent than medroxyprogesterone acetate (MPA), which has been widely used as a therapeutic agent for breast and endometrium cancers. FMPA was previously synthesized in 10 steps (total yield: 1%). An efficient synthesis of FMPA has been achieved in 6 steps (total yield: 12%). We examined the anti-tumor effect of FMPA, complexed with dimethyl-β-cyclodextrin (DM-β-CyD), on rat mammary carcinomas induced by 7,12-dimethylbenz[a]anthracene (DMBA). FMPA showed great anti-tumor effect on DMBA-induced rat mammary carcinomas.

Key words 9α-fluoromedroxyprogesterone acetate; synthesis; medroxyprogesterone acetate; anti-angiogenic agent; dimethylbenz[a]anthracene; mammary carcinoma

Neovascularization is involved in tumor growth as well as rheumatoid arthritis, diabetic retinopathy and age-related macular degeneration (AMD).1,2) Tumor growth is often accompanied by angiogenesis, the resulting blood vessels supplying nutrients necessary to tumor cells. The development of new blood vessels is also a crucial step in the invasion and metastasis of cancer cells. Breast cancer, melanoma, lung cancer, prostate cancer and other cancers.3) Thus, discovery of drugs which suppress angiogenesis has become one of the major focuses in cancer research. Angiostatin and endostatin, which are recombinant proteins, could be such drugs, but research on low-molecular inhibitors is still being vigorously studied in pharmaceutical industry.

As part of a research program to find novel anti-angiogenic drug candidates, we synthesized 9α-fluoromedroxyprogesterone acetate (FMPA, 17α-acetoxy-9α-fluoro-6α-methylprogesterone) (1) (Fig. 1).4) FMPA (1) is a fluorinated analog of medroxyprogesterone acetate (MPA) (2) (Fig. 1), which is widely used as a therapeutic agent for breast and endometrium cancer and possesses anti-angiogenic activity.

FMPA (1) exhibited an in vitro anti-angiogenic activity 100 times as strong as MPA (2) in a chorioallantoic membrane (CAM) assay system5) and exhibited a 7-fold stronger anti-angiogenic effect than MPA in a rabbit corneal assay system.5) In addition, FMPA (1), complexed with γ-cyclodextrin (γ-CyD), has shown anti-tumor effect on rat mammary carcinomas induced by 7,12-dimethylbenz[a]anthracene (DMBA) which is greater than the effect of the parent compound, MPA (2).5) FMPA (1) inhibits the activity of plasminogen activator (PA), an important protease playing an essential role in the process of angiogenesis, in bovine endothelial cells.5) However, further biological evaluations such as in vitro anti-tumor tests have been difficult, because the first synthesis method of FMPA (1) developed had low overall yield (1%) and required many steps (10 steps). To overcome these problems, a practical synthetic route to FMPA (1) which involves six-steps and has 12% overall yield has been newly established. We here describe the full details of both the synthesis of FMPA (1) and its anti-tumor effect on rat mammary carcinoma when complexed with dimethyl-β-cyclodextrin (DM-β-CyD).

Results and Discussion

For the synthesis of 9α-fluoro-MPA, FMPA (1), we utilized the 11β-hydroxy group of the steroid nucleus to introduce a fluorine atom at the 9α-position on both routes (Charts 1, 2). The initial route to 1 as shown in Chart 1 uses the commercially available 11β-17α-dihydroxy-4-pregnene-3,10-dione (3) as a starting material. Here, acetylation of the 11β-hydroxy group of 3 (100%) gave the compound 4. Sequential, acetalization and treatment with m-chloroperbenzoic acid (mCPBA) afforded the epoxide 6, which was reacted with CH3MgBr to yield the 5-hydroxy-6-methylpregnen-3. Treatment of 7 with 5% KHSO4 followed by treatment with 0.05 N NaOH afforded the 6-methylpregnen-9 (23% yield from 4). The fluorination of the 9α-position of 9 was carried out by 70% HF in pyridine5) to produce the 9α-
fluoroprogesterone 10 (23%). The 6-methylprogesterone 10 was converted into 6α-methylprogesterone 12 by acetalization followed by deacetalization in the usual manner8) (58% yield from 10). Finally, acetylation of 12 by the mixed anhydride method9) provided 9α-fluoromedroxyprogesterone acetate (1) (FMPA) (36%). The overall yield of the initial synthetic route was quite low (1%) in a ten-step sequence. On the other hand, our improved synthesis of FMPA (1) (Chart 2) was carried out starting from 6α-methylpredonisolone 13, which is a cheaper starting material than 11β,17α-dihydroxy-4-pregnen-3,10-dione 3. Specifically, acetylation of 13 was performed with trimethylorthoacetate, and then the resulting ortho ester was hydrolyzed to give 6α-methylpredonisolone 17α-O-acetate 14 (89%). The hydroxyl group at the C-21 position of 14 was converted into the mesylate, followed by treatment with sodium iodide to yield pregnadiene-3,20-dione 15 (59%). Reduction of 15 with hydrogen atmosphere in the presence of chlorotris(triphenylphosphine)rhomodium(I)10) provided 11β-hydroxy-pregnen-3,20-dione 16 (90%). Fluorination of 16 was performed using hydrogen fluoride in pyridine to produce FMPA (1) (26%). The structure of 1 was confirmed by spectroscopy. Thus, we succeeded in an efficient and practical synthesis of FMPA (1) with 12% overall yield in 6 steps.

FMPA (1) showed significant inhibitory effects on the growth of DMBA-induced rat mammary carcinomas at doses of 30 and 120 mg/kg as compared with a control group during the treatment and withdrawal periods (Fig. 2). The number of tumors which appeared after treatment was also significantly inhibited by FMPA (1) doses of 30 and 120 mg/kg compared with the control group (Fig. 3). It has been reported that FMPA (1) complexed with γ-CyD showed anti-tumor effect on DMBA-induced rat mammary carcinomas, and its effect is greater than that of the parent compound, MPA (2).5) The bioavailability of FMPA (1) complexed with γ-CyD was found to be about 6 times higher than that of FMPA (1) complexed with DMβ-CyD in rats. In a preliminary study of pharmacokinetics of FMPA (1) in rats, DMβ-CyD complex exhibited bioavailability about 2 times higher than γ-CyD complex (unpublished data), so we used FMPA (1) complexed with DMβ-CyD in this study. As a result, FMPA (1) exhibited strong anti-tumor activity on DMBA-induced rat mammary carcinomas. Therefore, it is suggested that oral ad-
ministration of FMPA (1) may be useful for treatment of mammary cancer.

**Conclusions**

In a preliminary work, it was proposed that the anti-angiogenic effect of MPA (2) might be increased by giving it a fluorine atom at the C-9α position, a novel design. Based on this hypothesis, two synthetic routes of FMPA (1) were found. The newer synthetic route is a practical process using a cheaper starting material. Subsequently, potent anti-angiogenic effects have been verified for the FMPA (1) in several bioassay systems. This study also indicates that FMPA (1) has strong anti-tumor activity, so that FMPA (1) may well be a useful oral drug for the treatment of human breast and endometrium cancer.

**Experimental**

**General**

Melting points were measured on a Yanagimoto micro-melting point apparatus MP-500D and were uncorrected. Proton nuclear magnetic resonance spectra (1H-NMR) were taken with a Varian Unity-500 instrument using tetramethylsilane as an internal standard. Mass spectra (MS) were determined by a Shimadzu QP5050 mass spectrometer. Elemental analysis was done by Yanaco CHN CORDER MT-6.

**11β-Acetoxyl-17α-hydroxy-4-pregnene-3,20-dione (4)** A mixture of 4-pregnene-11β,17α-diol-3,20-dione (3 g, 5.77 mmol), Ac2O (10 ml), pyridine (20 ml) and 4-dimethylaminopyridine (DMAP) (20 mg, 0.16 mmol) was stirred for 6 h at room temperature. The reaction mixture was diluted with water (50 ml) and extracted with CHCl3. The CHCl3 layer was sequentially washed with 10% HCl, a 5% aqueous NaHCO3 solution, and brine, dried over Na2SO4, and concentrated under reduced pressure. The residue was subjected to column chromatography (silica gel, 150 g) using 70% CHCl3/hexane as eluant to give the acetylated derivative 4 (2.24 g, 100%), mp 159.5—160 °C (EtOAc/hexane). 1H-NMR (CDCl3); δ 0.85 (3H, s), 2.01 (3H, s), 2.21 (3H, s), 3.04 (2H, m), 5.34—5.56 (1H, m), 5.65 (1H, s). MS m/z: 388 (M+). Anal. Calcd for C23H26O2: C, 71.10; H, 8.30. Found: C, 71.05; H, 8.50.

**6-Methyl-14β-hydroxy-11β,17α-diol-3,20-dione (9)** 11-Acetate 4 (2 g, 5.15 mmol), ethylene glycol (11.1 g, 17.7 mmol), and p-TsOH (40 mg, 0.21 mmol) were refluxed in benzene (100 ml) for 7 h while reducing humidity (by the use of a reflux condenser equipped with a moisture separator).

After the reaction temperature lowered to room temperature, the mixture was washed with brine, dried over Na2SO4, and concentrated. The residue was used directly in the next step without further purification. 70% CPBA (2 g, 8.11 mmol) was added to the residue 2 (150 ml), and the mixture was stirred for 12 h at room temperature. The reaction was sequentially washed with an aqueous Na2CO3 solution and brine, and then concentrated. The resultant residue 6 was thoroughly dried, and then dissolved in anhyd. THF (30 ml). While vigorously stirring the mixture, CH3MgBr (1.02 M THF, 30 ml, 30.6 mmol) was added. The reaction mixture was refluxed for 18 h, the temperature was lowered to room temperature, and the mixture was poured into ice-water containing NH4Cl. The mixture was extracted with CHCl3, and the organic layer was washed with saturated brine, dried over Na2SO4, and concentrated under reduced pressure. The residue 7 was refluxed at 70 °C for 2 h in a mixture of acetone (80 ml) and an aqueous 5% KHSO4 solution (40 ml). The organic layer was evaporated, and the residue was reextracted with CHCl3. The CHCl3 layer was washed with saturated brine, dried over Na2SO4, and concentrated under reduced pressure. Subsequently, the residue 8 was stirred for 2 h in a mixture of 0.05 N NaOH (100 ml) and MeOH (100 ml) at room temperature. Structures of crude compounds 5—8 were supported by the detection of main signals in 1H-NMR spectra, respectively. AcOH (1 ml) was added to the reaction mixture, and the mixture was concentrated under reduced pressure to ca. half volume. Extraction was performed with CHCl3. The CHCl3 layer was washed with brine, dried over Na2SO4, and concentrated under reduced pressure.

The residue was subjected to column chromatography (silica gel, 70 g) using 30% EtOAc/hexane as eluant to give the title compound 1 (388 mg, 32%), mp 115—120 °C (EtOAc). 1H-NMR (CDCl3): δ 1.03 (3H, s), 1.05 (3H, d, J = 6.9 Hz), 1.06 (3H, d, J = 7.57 Hz), 1.50 (3H, s), 2.30 (3H, s), 2.68—2.71 (2H, m), 3.48 (1H, q, J = 7.01 Hz), 4.47 (1H, d, J = 4.96 Hz), 5.73 (1H, s). MS m/z: 360 (M+). Anal. Calcd for C23H26O2: C, 73.70; H, 8.95. Found: C, 73.70; H, 8.99.

**9a-Fluoro-17α-hydroxy-6-methyl-4-pregnene-3,20-dione (10)** Hydrogen fluoride-pyridine [4 ml, HF: pyridine = 7:3 (w/v), 0.14 mol/l] was placed in a Teflon container. While maintaining the external temperature at −15 °C, under nitrogen, 11β-hydroxy-4-pregnene-3,20-dione (9) (200 mg, 0.55 mmol) was added. The mixture was stirred for 6 h at the same temperature, and the mixture was sequentially washed with 3% HCl, 5% NaHCO3, and brine, dried over Na2SO4, and concentrated under reduced pressure. The residue was subjected to column chromatography (silica gel, 30 g) using 30% EtOAc/hexane as eluant to give the fluorinated compound 10 (47 mg, 23.3%), mp 232—235 °C (EtOAc). 1H-NMR (CDCl3); δ 0.75 (3H, s), 0.78 (3H, s), 1.11 (3H, d, J = 6.35 Hz), 1.20 (3H, d, J = 4.96), 1.30 (3H, s), 1.37 (3H, s), 2.27 (3H, s), 2.28 (3H, s), 2.69—2.75 (2H, m), 2.99—3.07 (1H, m), 3.82 (1H, d, J = 1.84Hz), 5.85 (1H, d, J = 3.09 Hz), 5.90 (1H, d, J = 2.00 Hz), MS m/z: 225 (M+). Anal. Calcd for C24H26F2O2: C, 78.89; H, 8.62. Found: C, 72.95; H, 8.75.

**9β-Fluoro-17α-hydroxy-6-methyl-4-pregnene-3,20-dione (12)** The 6-methylpregnene 10 (40 mg, 0.11 mmol), ethylene glycol (0.014 ml, 0.25 mmol), and p-TsOH (2 mg, 0.016 mmol) were added to benzene (20 ml), and refluxed with heat for 3 h using a reflux condenser equipped with a moisture separator. The solvent was concentrated under reduced pressure, and the residue was combined with an aqueous 5% KHSO4 solution (5 ml) and acetone (5 ml). The mixture was refluxed for 2 h at 70 °C. The reaction mixture was concentrated under reduced pressure, and then the residue was extracted with CHCl3. The CHCl3 layer was washed with saturated brine,
dried over Na2SO4, and concentrated under reduced pressure. The residue was subjected to column chromatography (silica gel, 20 g) using 30% EtOAc/hexane as eluant to give the methylated derivative 12 (23 mg, 57.5% from compound 10), mp 237—239 °C (EtOAc). [α]D20 +23.76° (c = 0.202, MeOH). 1H-NMR (CDCl3): δ 0.86 (3H, s), 1.10 (3H, d, J = 6.45 Hz), 1.30 (3H, s), 2.69—2.72 (2H, m), 2.99—3.03 (1H, m), 3.84 (1H, d, J = 1.80 Hz), 3.89 (1H, d, J = 1.80 Hz). MS m/z: 362 (M+). Anal. Caled for C24H32O5: C, 71.97; H, 8.05. Found: C, 72.04; H, 8.78.

17α-Acetoxy-11β-hydroxy-6α-methyl-14,20-pregna-3,20-dione (16) Chlorotris(triphenylphosphine)rhodium(I) (8.51 mg, 9.20 μmol) was added to a solution of 17α-acetoxy-11β-hydroxy-6α-methyl-1,4-pregna-3,20-dione 15 (316 mg, 0.79 mmol) in CH2Cl2:EtOH (1:1; 30 ml). The mixture was stirred for 27 h at room temperature (20—25 °C) in a stream of hydrogen gas (1.5 kg/cm2). After completion of reaction, the reaction mixture was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (silica gel, 10 g) using 50% EtOAc/hexane as eluant to give 17α-acetoxy-11β-hydroxy-6α-methyl-1,4-pregna-3,20-dione 16 (285 mg, 90%), mp 193—195 °C (EtOAc). [α]D20 +55.4° (c = 0.773, CHCl3). 1H-NMR (500 MHz, CDCl3): δ 0.93 (3H, s), 1.06 (3H, d, J = 6.5 Hz), 1.43 (3H, s), 2.06 (3H, s), 2.07 (3H, s), 2.92—2.98 (1H, m), 5.73 (1H, d, J = 1.5 Hz). MS m/z: 402 (M+). Anal. Caled for C28H36O4: C, 71.61; H, 8.51. Found: C, 71.75; H, 8.53.

17α-Acetoxy-9α-fluoro-6α-methyl-14,20-pregna-3,20-dione (1) Hydrofluoride-pyridine [4 ml, HF: pyridine = 7:3 (v/w)] was placed in a Teflon container. While maintaining the exterior temperature at —15 °C, under nitrogen, 17α-acetoxy-11β-hydroxy-6α-methyl-4,20-pregna-3,20-dione 16 (145 mg, 0.36 mmol) was added. The solution was stirred for 65 h at the same external temperature of —15 °C. When the reaction was completed, ice water was added to the reaction mixture, which was then extracted with EtOAc (50 ml, 30 ml). The organic layer was sequentially washed with 10% HCl, saturated NaHCO3, and brine, and then dried over Na2SO4. After the solvent was evaporated under reduced pressure, the residue was subjected to column chromatography (silica gel, 6 g) using 40% EtOAc/hexane as eluant to give 17α-acetoxy-9α-fluoro-6α-methyl-14,20-pregna-3,20-dione (1) (37.9 mg, 26%), which was identical with the CMPA synthesized previously in all respects.

Anti-tumor Effect on Rat Mammary Carcinoma The following animal studies were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society. DBMA was administered orally at a single dose of 20 mg/body to female Sprague-Dawley rats about 50 days old. When the diameter of the first developed mammary tumor became 0.5—1.5 cm eight—12 weeks after DBMA administration, the animal was incorporated into the study. FMPA (1), complexed with DM-β-CyD, was administered to the animals orally once a day at doses of 30 and 120 mg/kg/day for 3 weeks. Distilled water and DM-β-CyD (498 mg/ml) were administered to the animals orally once a day at a volume of 6 ml/kg/day for 3 weeks. The two largest perpendicular diameters of each tumor were measured on the first day of treatment and thereafter once on every 2 weeks for the multiplied product of these diameters was expressed as tumor size. Number of tumors per rat appeared after treatment was also counted.

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References