Human Trophoblastic Cells Metabolize 25-Hydroxyvitamin D₃ *In Vitro*

Tetsuro OKABE, Seiichi ISHIZAKA and Michio FUJISAWA

Human trophoblastic cells (T3M-3) metabolized 25-hydroxyvitamin D₃ into two more polar metabolites during *in vitro* incubations. A two-step high pressure liquid chromatography system revealed two unique elution positions of those trophoblastic cell-derived metabolites that exactly co-migrated with the elution positions of 5(Z)-19-nor-10-oxo-25-hydroxyvitamin D₃ and 5(E)-19-nor-10-oxo-25-hydroxyvitamin D₃, respectively. These unique metabolites did not bind specifically to a protein receptor for 1,25-dihydroxyvitamin D₃.

Key words: Placenta, Vitamin D₃

It has been known that the placenta can synthesize 1,25-dihydroxy vitamin D₃[1,25(OH)₂D₃] (1). Why placenta produces 1,25(OH)₂D₃ is not known but it is presumably to supply the fetus. It is possible that the fetal kidneys are incapable of producing 1,25(OH)₂D₃ as suggested by Gray et al (2), because they are immature. However, it is also known that vitamin D-deficient mothers can give birth to normal babies, casting some doubt on this importance of 1,25(OH)₂D₃ to the fetus (3). Thus, involvement of the vitamin D₃ endocrine system with the fetal-placental units has now been recognized.

Choriocarcinoma is a neoplastic trophoblastic tumor which produces many of placental hormones including human chorionic gonadotropin, estrogens, and progesterone. Recently, we have established a functional trophoblastic cell line from a human choriocarcinoma (4). Since placental tissues have been shown to synthesize 1,25(OH)₂D₃, it may be of great interest whether the functional trophoblastic cells metabolize 25(OH)D₃ and which type of metabolite they produce. In this paper, we demonstrate that human trophoblastic cells (T3M-3) metabolize 25(OH)D₃ into more polar metabolites

during *in vitro* incubations.

MATERIALS AND METHODS

Cell culture

Human trophoblastic cell line, T3M-3, was continuously propagated in RPMI-1640 medium supplemented with 10% fetal bovine serum (Flow Laboratories Inc., Rockville, M.D.), penicillin (100 U/ml) and streptomycin (100 ug/ml) as described previously (4).

Incubations

The cells (1×10^6) were washed with RPMI-1640 medium and incubated with [26,27-methyl-³H] 25(OH)D₃ (Radiochemical Centre, Amersham, specific activity 22 Ci/mmol) for 60 min at 37°C in a humidified atmosphere of 5% CO₂ in air. The experiments were terminated by adding a volume of chloroform: methanol (1:1) to the cells and medium after centrifugation at 2,000 r.p.m. for 10 min.

Separation of [26,27-methyl-³H] 25(OH)D₃ metabolites by high pressure liquid chromatography

Cells and culture medium were extracted with two volumes of chloroform: methanol (1:1) twice. The chloroform phase was evaporated, and the residue was dried by ethanol azeotrope and chromato-

From The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo

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Reprint requests should be addressed to Tetsuro Okabe, MD, The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-Ku, Tokyo 113, Japan

graphed. The chloroform extracts from cells and/or medium were subjected to high pressure liquid chromatography on a Hitachi Model 635 equipped with a 4.5 × 250 mm Zorbax Sil column, and eluted with 10.5% isopropanol in n-hexane and 2.2% methanol in dichloromethane. The subtle structural differences among many vitamin D₃ are such that even high pressure liquid chromatography cannot achieve an unequivocal separation of all vitamin D₃ metabolites that may be present in the same sample with only one solvent system. Fractions were collected every 20 s from high pressure liquid chromatography column, and the radioactivity of the eluate was measured with a liquid scintillation counter.

Sucrose density gradient centrifugation analysis of receptor-metabolite complex

Chick intestinal cytosol 1,25(OH)₂D₃-specific receptor protein was prepared from vitamin D-deficient chicks as described previously (5). Aliquots (0.3 ml) of the intestinal cytosol (9.2 mg protein/ml) were incubated with 0.1 pmol of [26,27-methyl-³H] 1,25(OH)₂D₃ (158 Ci/mmol, 35,000 dpm) alone or with 0.45 pmol of [26,27-methyl-³H] 25(OH)D₃ metabolites peak A and peak B (both 22 Ci/mmol, 22,000 dpm) or with 100-fold excess of non-labelled 1,25(OH)₂D₃ at 25°C for 1 h. The aliquots (0.2 ml) of the cytosol were layered onto 5 ml of linear gradient of 5 to 20% (w/v) sucrose in 0.3 M KCl-phosphate buffer A (25 mM KH₂PO₄, 0.1 M KCl, 1 mM dithiothreitol, pH 7.4) and centrifuged at 0°C for 18 h at 216,400 × g (average force) using a Hitachi RP65 ultracentrifuge and RP65T rotor. Each 0.2 ml from the top of the centrifuge tube was collected in counting vials and the radioactivities were counted in 8 ml of Oxifluor-H₂O liquid scintillation solution (New England Nuclear) in a liquid scintillation counter. Sedimentation constants were estimated by comparison with standard protein markers, ovalbumin (3.67 S) and bovine serum albumin (4.4 S).

Compounds

25(OH)D₃, 24,25 (OH)₂D₃, natural 25(OH)D₃-26,23-lactone and 1,25(OH)₂D₃-26,23-lactone were synthesized at Teijin Institute for Bio-Medical Research. 5(E)-19-nor-10-oxo-25(OH)D₃ and 5(Z)-19-nor-10-oxo-25(OH)D₃ were synthesized from 25(OH)D₃ by osmium tetroxide oxidation and

then by periodate oxidation at Teijin Institute for Bio-Medical Research. [26,27-methyl-³H] 25(OH)D₃ (specific activity 22 Ci/mmol) were purchased from Radiochemical Centre, Amersham.

RESULTS

After incubation with [³H]-25(OH)D₃, material extracted from T3M-3 cells and subjected to high pressure liquid chromatography co-eluted with authentic 5(Z)- and 5(E)-19-nor-10-oxo-25(OH)D₃ (Fig. 1). In the lipid extracts of both T3M-3 cells and their incubation medium, there were three major radioactive peaks on high pressure liquid chromatography in 10.5% isopropanol in n-hexane. The first peak co-eluted with synthetic 25(OH)D₃, the substrate. The second peak co-migrated with 5(Z)-19-nor-10-oxo-25(OH)D₃ (Peak A metabolite). The third radioactive peak co-chromatographed with 5(E)-19-nor-10-oxo-25(OH)D₃ and 1,25(OH)₂D₃ (Peak B metabolite).

Peak A and Peak B metabolites were collected and rechromatographed using a different solvent system to verify the co-elution of these metabolites with 5(Z)- and 5(E)-19-nor-10-oxo-25(OH)D₃,

Fig. 1. Elution pattern of radioactivity from extracted T3M-3 cells and medium indicated by histogram and synthetic vitamin D₃ metabolites (absorbance at 260 nm and 310 nm) indicated by dotted line peaks in 10.5% isopropanol in n-hexane.

respectively. The second system involved a substitution of 2.2% methanol in dichloromethane for the 10.5% isopropanol in n-hexane. Peak A metabolite co-eluted with 5(Z)-19-nor-10-oxo-25(OH)D₃ also on this solvent system (Fig. 2). Peak B metabolite did not co-migrate with 1,25(OH)₂D₃ on this system

Fig. 2. Elution pattern of radioactivity obtained when Peak A metabolite in 10.5% isopropanol in the n-hexane system was re-chromatographed in 2.5% methanol in the dichloromethane system.

Fig. 4A. Elution patterns of the synthetic vitamin D₃ metabolites in 10.5% isopropanol in the n-hexane system.

but exactly co-chromatographed with 5(E)-19-nor-10-oxo-25(OH)D₃ (Fig. 3). These elution posi-

Fig. 3. Elution pattern of radioactivity obtained when Peak B metabolite in 10.5% isopropanol in the n-hexane system was re-chromatographed in 2.2% methanol in the dichloromethane system.

Fig. 4B. Elution patterns of the synthetic vitamin D₃ metabolites in 2.2% methanol in the dichloromethane system.

tions did not match those of other vitamin D₃ metabolites available in this laboratory, including natural 25(OH)D₃-26,23-lactone and 1,25(OH)₂D₃-26,23-lactone, except 19-nor-10-oxo-25(OH)D₃ (Fig. 4).

In an attempt to functionally characterize the tritiated metabolites, Peak A and Peak B metabolites were employed as radiolabelled ligands for high affinity intracellular binding protein (receptor) for 1,25(OH)₂D₃. [³H]-1,25(OH)₂D₃ was completely displaceable by 100-fold excess of unlabelled 1,25(OH)₂D₃. However, Peak A or Peak B metabolite did not bind specifically to the 1,25(OH)₂D₃ specific 3.7S receptor protein (data not shown).

DISCUSSION

The results presented in this paper show that human trophoblastic cells (T3M-3) metabolize 25(OH)D₃ into two more polar derivatives. The metabolites obtained behaved like 5(Z)- and 5(E)-19-nor-10-oxo-25(OH)D₃, respectively, on Zorbax sil column chromatography with two different solvent systems. We have also noticed the conversion of these metabolites upon prolonged handling (Figs. 2, 3), which is characteristic to isomers of 5(Z)- and 5(E)-19-nor-10-oxo-25(OH)D₃ (6). From these observations, the metabolites synthesized by T3M-3 cells appear to be 5(Z)- and 5(E)-19-nor-10-oxo-25(OH)D₃.

Recently, it has been shown by several investigators that placental tissues can synthesize 1,25(OH)₂D₃ (1-3). Together with their results, our observations raise the possibility that trophoblastic cells play an important role in vitamin D₃ metabolism in the fetal-placental unit.

The role of these metabolites is not known. These metabolites were first isolated after incubation of

25(OH)D₃ with bovine rumen microbes (6). The preliminary observations suggested that these compounds did not stimulate bone calcium mobilization and they were functionally inactive. In the present experiments, these metabolites did not specifically bind to 1,25(OH)₂D₃ specific receptor protein (data not shown) supporting the claim that these compounds are functionally inactive. It appears that these functionally inactive compounds are in the pathway of vitamin D₃ deactivation. The present observations may raise the possibility that these trophoblastic cells are able to control the level of substrate, 25(OH)D₃ for 1 α -hydroxylase, thereby reducing the formation of the active metabolites of vitamin D₃, 1,25(OH)₂D₃ in the fetal-placental unit (1-3). This interesting possibility of negative feedback regulation of vitamin D₃ metabolism in the fetal-placental unit raises a hypothesis for future study.

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