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INFORMATION

Hokkaido University confered the degree of Doctor of Philosophy (Ph. D) in Veterinary Medicine on September 25, 1998 to 2 recipients and December 25, 1998 to 2 recipients.

The titles of their theses and other information are as follows:

24R, 25-Dihydroxyvitamin D_3: a vitamin D_3 metabolite essential for the healing process of a fracture and the evidence for its membrane receptor in fracture healing tissue

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24R, 25-dihydroxyvitamin D_3 [24R, $25(OH)_2D_3$] is one of the metabolites of vitamin D_3 produced in the kidney. In spite of its existence in high concentrations in plasma, the biological function of 24R, $25(OH)_2D_3$ is not certain yet. On the basis of several reports suggesting its involvement in bone biology, I have investigated the possible biological role of 24R, $25(OH)_2D_3$ in bone, particularly in a fracture-healing model in chicks.

First the effect(s) of 24R, $25(OH)_2D_3$ on fracture-healing was studied in a vitamin D-depleted chick model. 24R, $25(OH)_2D_3$, together with another hormonally active vitamin D metabolite, 1α,25-dihydroxyvitamin D_3 [1α, $25(OH)_2D_3$], improved bone mechanical strength parameters (torsional strength, angular deformation, and stiffness) and the ash content. The synthetic epimer 24S, 25-dihydroxyvitamin D_3 [24S, $25(OH)_2D_3$] was not as potent as the natural 24R, $25(OH)_2D_3$. The administration of 24S, $25(OH)_2D_3$ combined with 1α, $25(OH)_2D_3$ or 1α, $25(OH)_2D_3$ alone resulted in poor healing compared to birds treated with 24R, $25(OH)_2D_3$ plus 24R, $25(OH)_2D_3$ or the vitamin D_3 control group.

In light of the ability of the fracture-healing callus to discriminate between 24R, $25(OH)_2D_3$ and 24S, $25(OH)_2D_3$, I explored the presence of a specific 24R, $25(OH)_2D_3$ receptor in fracture-healing callus tissue. No evidence was obtained for a classical nuclear/cytosol receptor for 24R, $25(OH)_2D_3$ in the fracture-healing callus. A specific receptor/binding protein for 24R, $25(OH)_2D_3$ was found in the callus membrane fraction, which showed ligand specific binding with dissociation constant (K_D) of 18.3 ± 1.9 nM, the maximal binding site (B_{max}) of 41.9 ± 6.0 fmol/mg protein and Relative Competitive Index (RCI) for 24R, $25(OH)_2D_3$/24S, $25(OH)_2D_3$/$25(OH)D_3$/1α, $25(OH)_2D_3$ of 100/37/42/1.8. The RCI pattern was different from that of chick serum vitamin D binding protein (DBP) (RCI= 100/100/219/4.3) which did not differentiate between epimeric isomers. Whereas, the membrane receptor for 24R, $25(OH)_2D_3$ exhibited high specificity for 24R, $25(OH)_2D_3$, distinguishing small structural differences among epimeric isomers, 24S, $25(OH)_2D_3$ and 24R, $25(OH)_2D_3$

Previous studies have implicated the biological roles of two vitamin D metabolites, 1α, $25(OH)_2D_3$ and 24R, $25(OH)_2D_3$ in the process of skeletal fracture-healing. While a nuclear re-

ceptor for 1α, $25(OH)_2D_3$ is known to be present in osteoblast and absent in osteoclast cell lines, no systematic study has been carried out on the callus tissue which is formed during fracture-healing. Therefore I investigated a 1α, $25(OH)_2D_3$ receptor/binding protein for all cullus fractions: nuclear, postnuclear membrane, and high speed cytosol fraction of the callus tissue of a tibial fracture. The binding of 1α, $25(OH)_2D_3$ observed in the nuclear fraction was not saturable. Saturable binding was observed in the callus membrane and the cytosol fractions where the K_D/B_{max} values were 0.83 ± 0.35 nM/35.8 ± 5.28 fmol/mg protein and 0.66 ± 0.38 nM/9.8 ± 1.4 fmol/mg protein, respectively. These receptor-ligand kinetics values were clearly different from those of the membrane receptor for 24R, $25(OH)_2D_3$.

Thus I confirmed the presence of a membrane binding protein for 24R, $25(OH)_2D_3$, which is distinct from the 1α, $25(OH)_2D_3$ receptor and also from DBP. This implies that 24R, $25(OH)_2D_3$ may generate biological responses via a signal transduction pathway(s) separate and distinct from that of 1α, $25(OH)_2D_3$. Collectively, my results suggest that 24R, $25(OH)_2D_3$ is a functionally important vitamin D_3 metabolite in bone biology and may function to generate biological responses through interaction with the membrane receptor indicated in the present study.

Original papers of this thesis appeared in "Biochem. Biophys. Res. Commun." Vol. 244, 724–727 (1998), and "Bone" Vol. 23(2), 141–146 (1998).

Environmental Monitoring Using Wildlife as a Biomarker: Inhabiting Environment Differentially Changes P450 Isozyme Specific Activities in Wild Rodents

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Summary

In order to estimate the suitability of using accumulation of pollutants in wildlife as an indicator of environmental pollution, I investigated the residue levels of organochlorine compounds (OCs) and their accumulation patterns in 8 species of terrestrial mammals and 10 species of birds. The accumulation of OCs to environment has been of great concern, because of their persistent and less degraded properties.

OCs accumulated in terrestrial mammals and birds were mostly in the order of polychlorinated biphenyls (PCBs)>dichlorodiphenyltrichloroethane compounds (DDTs)>hexachlorocyclohexane isomers (HCHs)>hexachlorobenzene (HCB). The accumulation levels of OCs in terrestrial mammals were lower than those in birds. The contamination levels of OCs were found to be higher in omnivorous mammals than in herbivorous ones, and in fish-eating ones and