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The tissue-specific distribution of ^{3}H-seocalcitol (EB 1089) and ^{3}H-calcitriol in rats☆

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Abstract

Seocalcitol (EB 1089) is under development for the treatment of hepato-cellular carcinoma (HCC). The tissue distribution of ^{3}H-seocalcitol was investigated in comparison to ^{3}H-calcitriol in rats. Quantitative whole-body autoradiography was used to quantify the tissue distribution. The greatest difference in distribution between the two compounds was observed in the bloodstream. For most tissues the ratio seocalcitol/calcitriol varied between 0.2 and 3.1. The concentration of radioactivity in the liver was almost the same for the two compounds. For seocalcitol the concentration in the liver was 10 times higher than in serum. Assuming that the liver/serum concentration ratio is the same in rats and humans, the concentration of seocalcitol in the human liver is expected to be higher than the concentration resulting in more than 50% inhibition of cancer cell proliferation, and thus pharmacologically effective in HCC. It is questionable whether calcitriol would be present in the human liver in sufficient concentrations to be effective for the treatment of HCC, as the antiproliferative activity of calcitriol is generally more than 10-fold lower compared to that of seocalcitol and as calcitriol can only be administered at a dose that is ca. three-fold lower than the dose of seocalcitol.

Keywords: Seocalcitol/EB 1089; Calcitriol; Tissue distribution; Rats

1. Introduction

Seocalcitol [1(S),3(R)-dihydroxy-20(R)-(5′ethyl-5′-hydroxy-hepta-1′(E),3′(E)-dien-1′-yl)-9,10-secopregna-5(Z),7(E),10(19)-triene] (EB 1089) is an analog of 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], the physiologically active form of vitamin D_3 (Fig. 1). 1α,25$(OH)_2D_3$ plays a crucial role in the regulation of calcium homeostasis [1] and in cell-growth regulation [2]. The effects of 1α,25$(OH)_2D_3$ are believed to be mediated via a specific intracellular receptor found in many cells and tissues, and they are not restricted to the regulation of calcium metabolism [3]. The clinical usefulness of 1α,25$(OH)_2D_3$ is primarily limited by its effect on calcium homeostasis, with the risk of inducing hypercalcemia and soft tissue calcifications. In contrast, the synthetic analog seocalcitol has been found to exert pronounced antiproliferative effects both in vitro and in vivo, but with reduced effects on calcium metabolism [4–7]. Seocalcitol is presently under clinical evaluation for the systemic treatment of hepacellular carcinoma (HCC) [8]. The concentration of seocalcitol in the liver is a key factor to obtain efficacy in HCC.

Previous experiments have suggested that seocalcitol has a tissue-specific uptake different from that of calcitriol. Though the serum half-lives of seocalcitol and calcitriol are comparable, their initial/apparent volume of distribution is very different, which is demonstrated by their different serum concentration after intravenous administration of the same dose to rats [9]. This is explained by the difference in their binding affinity for the vitamin D-binding protein (DBP) for which calcitriol has a 30-fold higher binding affinity. The distribution of the two compounds in rats in more than 30 organs/tissues is investigated using quantitative whole-body autoradiography (QWBA). The total radioactivity concentration in the liver of the two compounds is compared to their pharmacologically active concentration.

2. Materials and methods

2.1. Compounds

[1α-^{3}H]-Seocalcitol (28.7 μg/mL; 31.5 MBq/mL) and [1α-^{3}H]-calcitriol (50 μg/mL; 51 MBq/mL) were synthe-

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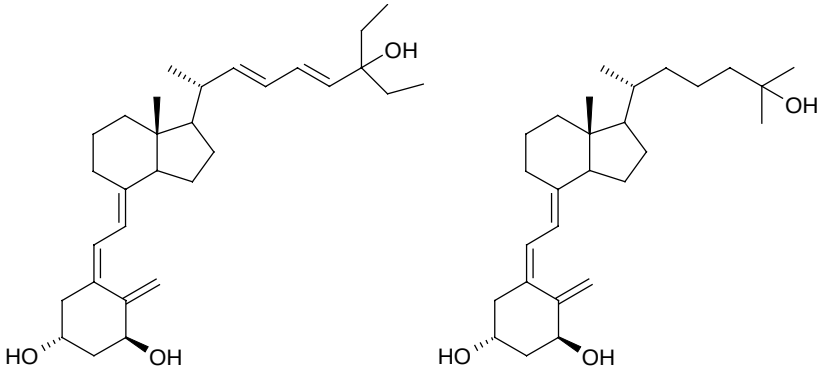

Fig. 1. Chemical structure of secalcitol (EB 1089) (I) and calcitriol (1α,25(OH)$_2$D$_3$) (II).

sized at LEO Pharma. The compounds were dissolved in a buffered aqueous solution suitable for intravenous administration.

2.2. Animals

Lister-Hooded (pigmented) male rats were obtained from Charles River, UK. The rats weighed ca. 230 g and were ca. 6 weeks old at dosing. The animals had free access to water and feed at all times.

2.3. Dosing and sampling

The rats were dosed by bolus injection with 47.5 μg/kg seocalcitol or 50 μg/kg calcitriol. Two and 8 h after administration single animals were sacrificed. The animals were plunged into a freezing mixture containing an excess of dry-ice in heptane. All carcasses were retained in the freezing mixture for a minimum of 30 min.

2.4. Whole-body autoradiography

The legs, tail and whiskers were trimmed off and each frozen carcass was set in a block of frozen aqueous 2% (w/v) carboxymethyl cellulose. The block was mounted onto the stage of a cryomicrotome (e.g. a CM3600 NT from Leica Microsystems, Milton Keynes, UK) maintained at about −20 °C. Sagittal sections were obtained through the carcass. The sections were freeze-dried and afterwards placed in contact with FUJI imaging Plates (Raytek Scientific, Sheffield, UK). After exposure in a refrigerator for 14 days, the imaging plates were processed using a FUJI BAS 1500 radioluminography system (Raytek Scientific). Electronic images were analysed using a PC-based image analysis package (Seescan Densitometry software, LabLogic, Sheffield, UK). ^{3}H-blood standards included with each autoradiogram were used to construct calibration lines over a range of radioactivity concentrations. The concentration of radioactivity in the tissue was calculated as nanogram seocalcitol or calcitriol equivalents per gram of tissue.

3. Results

The tissue distribution of [^{3}H]-seocalcitol and [^{3}H]-calcitriol was quantified in more than 30 organs/tissues. The radioactivity concentration in the clinically most relevant organs is given in Table 1. The greatest difference in the tissue distribution between the two compounds was observed in the bloodstream, which is explained by the difference in the binding affinity for DBP. For most tissues the seocalcitol/calcitriol ratio varied between 0.2 and 3.1. In more than 50% of the more than 30 organs/tissues investigated, the seocalcitol/calcitriol ratio was >1. As illustrated in the autoradiogram in Fig. 2, seocalcitol is widely distributed in the body with the highest concentration of radioactivity found in the liver. In rats dosed with calcitriol at the 2 h sampling time, the radioactivity was 3–2 times higher in the blood and the lung, respectively, than in the liver. But at the 8 h sampling time the radioactivity was slightly higher in the liver than in the blood.

4. Discussion

The findings in the present study are in accordance with the results obtained in a previous study in which seocalcitol was administered orally to both rats and minipigs and in which the liver/serum concentration ratio of intact seocalcitol was approximately 10 [10]. No distribution studies of seocalcitol have yet been performed in humans. However, after oral administration of a single clinically relevant dose of seocalcitol (15 μg) the maximal serum concentration of seocalcitol in 84 human volunteers was 57 pg/mL [LEO Internal Report]. Assuming that the liver/serum concentration ratio of seocalcitol in humans is similar to the one in rats and minipigs, the maximal concentration

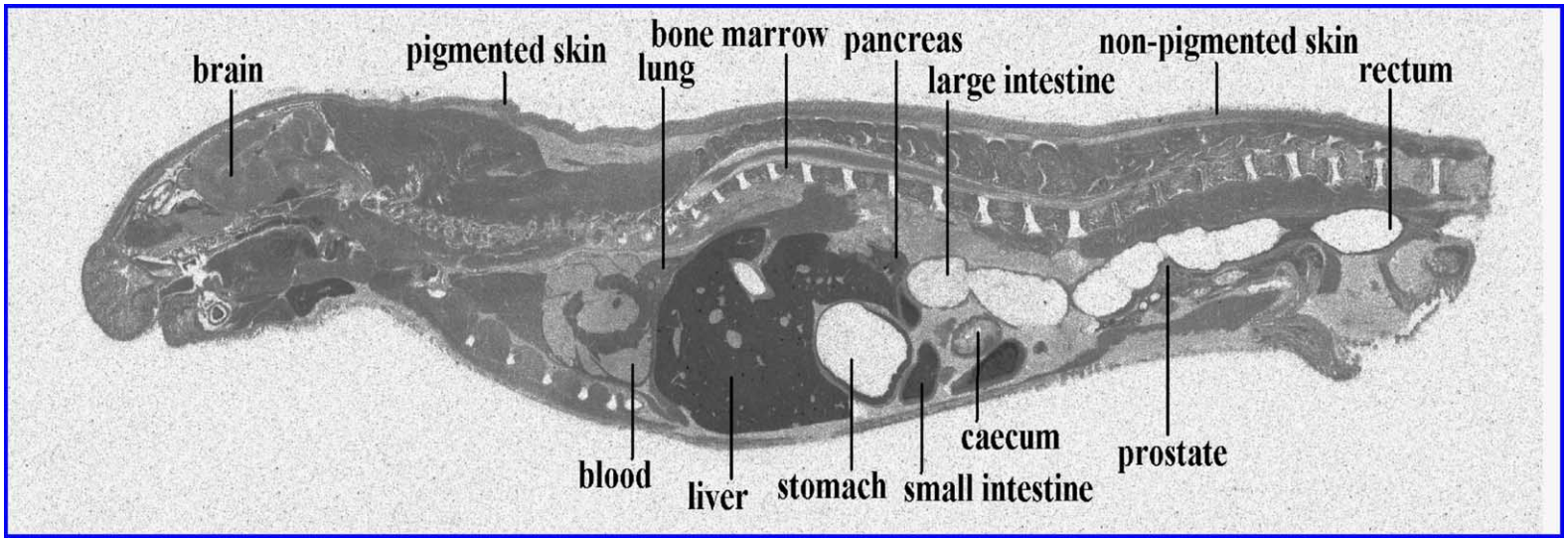

Fig. 2. The tissue distribution of [^{3}H]-seocalcitol 2 h after intravenous administration to a pigmented male rat.

Table 1
Concentrations of radioactivity in the tissues of male pigmented rats after a single intravenous administration of [^{3}H]-seocalcitol or [^{3}H]-calcitriol

Tissue	Nanogram equivalents seocalcitol per gram tissue		Nanogram equivalents calcitriol per gram tissue		Ratio: seocalcitol/calcitriol	
	2 h	8 h	2 h	8 h	2 h	8 h
Blood	23	14	281	79	0.08	0.18
Lung	65	34	196	46	0.3	0.7
Liver	153	125	106	121	1.4	1.0
Kidney (cortex)	56	37	84	46	0.7	0.8
Kidney (medulla)	53	33	114	32	0.5	1.0
Pancreas	100	33	45	15	2.2	2.2
Small intestine	58	53	29	88	2.0	0.6
Large intestine	19	41	22	18	0.9	2.3
Prostata	36	29	66	27	0.5	1.1
Muscle	43	15	33	11	1.3	1.4
Brain	36	12	10	<10	3.6	>1
Skin (non-pigmented)	25	8	30	14	0.8	0.6
Skin (pigmented)	35	9	42	20	0.8	0.5
Bone marrow	35	18	30	11	1.2	1.6

The doses are normalized to 50 μg/kg.

Table 2
Effects of seocalcitol (EB 1089) on tumor cell proliferation in vitro

Test Compound	Cell line	Inhibition of proliferation IC_{50} (M)
Seocalcitol (EB 1089)	MCF-7 (human breast cancer)	2×10^{-10}
Calcitriol (1α,25(OH)$_2$D$_3$)		1×10^{-8}
Daunomycin		2×10^{-8}
Seocalcitol (EB 1089)	HT-29 (human colon cancer)	8×10^{-10}
Calcitriol (1α,25(OH)$_2$D$_3$)		4×10^{-8}
Daunomycin		8×10^{-9}
Seocalcitol (EB 1089)	B16 (mouse melanoma)	6×10^{-11}
Calcitriol (1α,25(OH)$_2$D$_3$)		6×10^{-9}
Daunomycin		5×10^{-8}

Source: See [11].

of seocalcitol in the human liver is above 10^{-9} M (~450 pg/g). As the IC_{50} for the antiproliferative effect of seocalcitol in many cancer cells is 10^{-10} to 10^{-9} M (Table 2), it is expected that seocalcitol is available at the target site (liver) at a sufficiently high concentration and duration to exert a pharmacological effect. This theory is further confirmed by the results from a phase II study in HCC. Out of 33 patients evaluable for tumor response, two complete responses and 12 cases of stable disease were reported [8].

It is questionable whether calcitriol would be present in the human liver at sufficiently high concentrations to be effective for the treatment of HCC. The two major reasons for this are that the antiproliferative activity of calcitriol is generally more than 10-fold lower compared to that of seocalcitol and that calcitriol can only be administered at a dose that is ca. three-fold lower than the dose of seocalcitol because of its hypercalcemic effects.

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