Materials (Data)

The Studies of *in Vivo* Distributions of Radioiodinated Cobalt-bleomycin in Tumor-bearing Animals by the Whole Body Autoradiography

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High tumor uptake of cobalt-bleomycin suggested that it would be a suitable starting material for chemical modification study. We already described the synthesis of 3-¹²⁵I-iodobenzyl cobalt-bleomycin and its in vivo distributions in tumor-bearing animals. In the present study, it was shown by the whole body autoradiogram that 3-¹²⁵I-iodobenzyl cobalt-bleomycin accumulated in tumor (dorsum, thigh, neck), the kidney, the liver and feces. Tumor affinity of this compound was proved by the results of the whole body autoradiogram.

Key Words: cobalt-bleomycin, iodine-125, scid mouse, malignant tumor, whole body autoradiography

1. Introduction

It was reported by Umezawa et al.¹⁾ that the bleomycins were a group of antibiotics and their therapeutic effect against carcinoma was proved by clinical studies. ⁵⁷Co-bleomycin which is produced by binding radioactive cobalt (Co-57) to bleomycin accumulates selectively in cancer and the excellent clinical results obtained with this compound.^{2–7)} ⁵⁷Co-bleomycin was introduced for tumor scanning by Nouel et al.⁴⁾ and by Kono et al.²⁾ and by Maeda et al.³⁾ in 1972, and has been used mainly in Europe

and Japan. Suzuki et al.⁷⁾ described their clinical experience with ⁵⁷Co-bleomycin in various tumors and discussed its clinical significance. Tumor scanning with ⁵⁷Co-bleomycin was carried out in 46 patients with an overall accuracy of 66% in malignant tumors. There were no genuine false positive results in four benign lesions. Since ⁵⁷Co-bleomycin is excreted rapidly through the kidneys, a high tumor-to-blood ratio is obtained a short time after injection. Although its long physical half-life may restrict its routine use, ⁵⁷Co-bleomycin might provide a valuable adjunct in the work-up of malignant tumors

when used in properly selected patients.

However, the long physical half-life of ^{57}Co (270 days) prevented its widespread clinical use.

High tumor uptake of cobalt-bleomycin suggested that it would be a suitable starting material for chemical modification studies.

Our study aimed at labeling cobalt-bleomycin with radioactive iodine and at clarifying its tumor-localizing property, because this element has several nuclides which are suitable for SPECT (^{123}I), PET (^{124}I), radiation therapy (^{131}I), and basic research (^{125}I).

We already described the synthesis of 3- ^{125}I -iodobenzyl cobalt-bleomycin (3- ^{125}I -Bz-Co-BLM) and its *in vivo* distributions in tumor-bearing animals.⁸⁾ In this paper, we show the whole body autoradiogram of tumor-bearing mice which were injected with 3- ^{125}I -Bz-Co-BLM.

2. Materials and methods

2.1 Materials

Bleomycin A₂ sulfate was given by NIPPON KAYAKU Co., LTD. (Japan). We purchased Iodine-125 (as Na ^{125}I) from Perkin Elmer life and analytical sciences (USA), 3-iodobenzylalcohol and diphosphorus tetraiodide (P₂I₄) from Sigma-Aldrich com (USA), cobalt chloride hexahydrate, citric acid monohydrate, 2,5-dihydroxybenzoic acid, copper sulfate pentahydrate and tin chloride dihydrate from Wako Pure Chemical Industries, Ltd (Japan) and male scid mice from Clea Japan, Inc. (Japan). Male scid mice (eight weeks old) were implanted with Ehrlich tumor (*ca.* 4×10^7 cells) in the thigh and the dorsum and used for these experiments six days later.

2.2 Methods

3- ^{125}I -Bz-Co-BLM: synthesized by the method described by us.⁸⁾

3- ^{125}I -Bz-Co-BLM was prepared for the injection reagent to mice. This solution (0.3 mL) contained about 11 kBq and 40 μg of 3- ^{125}I -Bz-Co-BLM.

3- ^{125}I -Bz-Co-BLM injection reagent (0.3 mL) was administered intravenously through the tail vein of the above tumor-bearing mice, respectively. These mice ($n=3$, weight 23.5–25.7 g) were sacrificed under carbon dioxide from dryice at three hours after the administration.

Sacrificed mice were frozen rapidly in acetone cooled to -70°C with dryice. The frozen mice were embedded in a gel of carboxymethyl cellulose sodium salt, and these mounted mice were frozen again in a similar manner. Sagittal sections (40 μm) through the whole frozen mice were taken from the tissues of interest with a cryomicrotome (Autocryotome NA-500F Nakagawa seisakusho) at -20°C . These frozen sections were dried at -20°C , and then exposed to an imaging plate (FUJIFILM FLA-700) for four weeks.

3. Results and discussion

In three mice administered with 3- ^{125}I -Bz-Co-BLM, very similar results were obtained. Fig. 1 illustrates the results of a typical mouse.

Fig. 1(A) shows the sagittal section of a mouse administered with 3- ^{125}I -Bz-Co-BLM, and tumor, liver, kidney, lung and feces are shown in this Figure by arrows.

Fig. 1(B) shows the whole body autoradiogram of this sagittal section. This indicates that radioactivities accumulate in the tumor (dorsum, thigh), the kidney, the liver and feces. In the tumor tissue, the accumulation of radioactivity in the viable tumor tissue was much more than in the necrotic tumor tissue.

In the Fig. 1(A), unexpectedly, it was found that small tumors (indicated by four small arrows) grew in the neck after the transposition of some tumor cells from the dorsum.

As shown in Fig. 1(B), radioactivities (indicated by four small arrows) accumulated in these small tumors.

Of ^{57}Co -bleomycin, Kono et al.^{5, 6)} described that ^{57}Co -bleomycin accumulated in the tumor tissue and

Fig. 1 Illustrates the results of a typical mouse. (A) shows the sagittal section of a mouse administered with $3\text{-}^{125}\text{I-Bz-Co-BLM}$. (B) shows the whole body autoradiogram of this sagittal section. This indicates that radioactivities accumulated in tumor (dorsum, thigh, neck), the kidney, the liver and feces (Color online).

this compound located only in the viable tumor tissue and was not seen in the necrotic tumor tissue, and that ^{57}Co -bleomycin bound to DNA in tumor cell.

From the results by Kono et al.,^{5, 6} by us⁸) and the present study, we presume that $3\text{-}^{125}\text{I-Bz-Co-BLM}$ accumulates in the viable tumor tissue and binds to DNA in tumor cell.

We already reported that the accumulation of $3\text{-}^{125}\text{I-Bz-Co-BLM}$ in tumor tissue was much more than other normal organs except the kidney and the liver.⁸⁾ The present study illustrates the results described above. This fact that this compound had considerably strong affinity for malignant tumor was proved by the whole body autoradiography. Unexpectedly, small tumors which grew in the neck after the transposition from the dorsum were confirmed by the whole body autoradiography.

We are planning to observe the distribution of

$3\text{-}^{125}\text{I-Bz-Co-BLM}$ in the tumor tissue by microautoradiography and to scan tumor-bearing animals after intravenous injection of $3\text{-}^{125}\text{I-Bz-Co-BLM}$.

4. Conclusion

It was shown by the whole body autoradiogram that radioactivities accumulated in the tumor (dorsum, thigh, neck), the kidney, the liver and feces. Tumor affinity of $3\text{-}^{125}\text{I-Bz-Co-BLM}$ was proved by the whole body autoradiogram.

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和文要旨

全身オートラジオグラフィによる放射性ヨウ素標識コバルト-プレオマイシンの担がん動物における体内分布の研究

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コバルト-プレオマイシンは腫瘍に多く取り込まれるので, この化合物は化学的に修飾するための出発物質に適している。我々は既に, $3\text{-}^{125}\text{I}$ -コバルト-プレオマイシンの合成とこの化合物の担がん動物における体内分布を報告した。今回の研究で, $3\text{-}^{125}\text{I}$ -コバルト-プレオマイシンが腫瘍(背, 大腿皮下, 首), 腎臓, 肝臓, 糞に多く集積することが全身オートラジオグラムによって示された。この化合物の腫瘍親和性が全身オートラジオグラムによって証明された。

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