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Tumor Visualization by ^{99m}Tc-Labeled Ethylenediamine-N,N-diacetic Acid[1)]

Tumor tissues were clearly visualized in scintigrams of mice bearing Ehrlich ascites tumor a few hours after the administration of ethylenediamine-N,N-diacetic acid labeled with ^{99m}Tc.

Keywords——radiopharmaceuticals; tumor scintigraphy; ^{99m}Tc; ethylenediamine-N,N-diacetic acid; Ehrlich ascites tumor

Among many radiodiagnostic agents developed for the scintigraphic visualization of tumors, clinically favored are ^{67}Ga, ^{201}Tl, and bleomycin labeled with various radionuclides.[2)] Though they are effective in imaging certain types of tumors, specific scanning agents are not available for a number of other malignant diseases. ^{99m}Tc is an ideal radionuclide for the scintigraphy because of its favorable physical characteristics and is widely used clinically for the scanning of various organs.

We have been working on ^{99m}Tc labeled amino acids to evaluate them as radiopharmaceuticals.[1)] In the course of the study, we found that ^{99m}Tc radioactivity was concentrated in Ehrlich ascites tumors after the administration of ethylenediamine-N,N-diacetic acid (EDDA) labeled with ^{99m}Tc to experimental animals. This communication presents the *in vivo* behavior and the scintigram of ^{99m}Tc EDDA and suggests that it can be a new radiodiagnostic agent for tumor visualization.

EDDA was prepared by the reported method[3)] and labeled with ^{99m}Tc by the $SnCl_2$ method as described previously.[1)] The labeling yield was greater than 99% when estimated by the thin layer chromatography. The ^{99m}Tc EDDA complex thus prepared was stable in neutral saline solution, other radiochromatographic peak being undetectable on standing it for 24 h at room temperature.

A group of mice (male; 30±2 g body weight) were implanted with Ehrlich ascites tumor cells at the right foreleg. The mice were left for 3 weeks for the growth of the tumors. A saline solution of ^{99m}Tc EDDA (0.1 ml, 500 μCi) was injected through the tail vein in the mice showing tumor growth. The animals were sacrificed 5 h after the injection. The organs, blood, some muscle, and the tumor were removed, weighed, and the radioactivity was measured with an autogamma scintillation spectrophotometer (Packard 5360). The percentages of the injected dose per gram of organs and tissues were calculated.

The results were blood, 0.19±0.12; liver, 1.90±0.46; spleen, 1.12±0.21; stomach 0.41±0.22; intestine, 1.25±0.16; kidney, 12.61±2.41; muscle, 0.38±0.11; and tumor, 4.15±1.01. The values are mean ±SD of three animals.

Very high uptake of radioactivity by the tumor and the kidneys is noted. The tumor/blood and tumor/muscle ratios were 4,61 and 10.92, respectively, 5 h after the injection.

These ratios are quite satisfactory for scintigraphic visualization of tumors. *In vivo* behavior of ^{99m}Tc EDDA was studied previously with dogs, rabbits, and golden hamsters that did not bear tumors.[1] ^{99m}Tc activity was not accumulated in any specific organ and excreted through kidneys. The high radioactivity uptake by the kidneys indicates the urinary excretion of ^{99m}Tc EDDA.

Scintigraphic studies were carried out on mice bearing Ehrlich ascites tumor after an intravenous injection of ^{99m}Tc EDDA (500 μCi in 0.1 ml saline). Sequential scintigrams were made at 1-h intervals for 5 h using a scintillation camera (Toshiba GCA 202) with a pinhole collimator. Total count in each scintigram frame was 1.5×10^5.

The image of the tumor was recognized on the scintigram in 1 h after administration, and visualized very clearly 2—5 h after the administration. Fig. 1 is a scintigram taken 4 h after the injection of ^{99m}Tc EDDA. The thyroid grand, mouth, stomach, and intestine were not imaged. This suggests that ^{99m}Tc pertechnetate was not liberated *in vivo*. Since the high radioactivity was found in the kidneys, scintigraphic visualization of tumors in this neighborhood would be prevented.

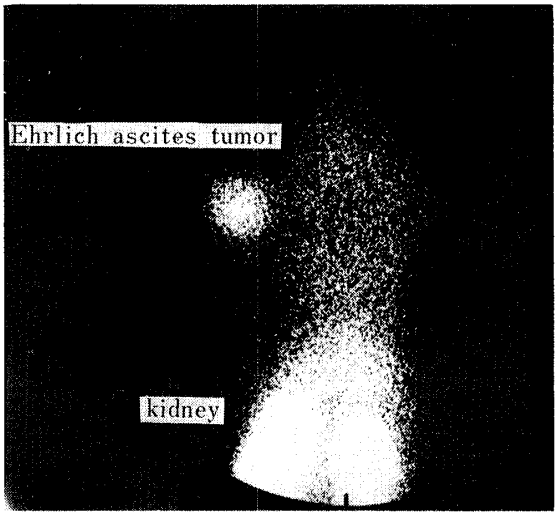

Fig. 1. A Scintigram obtained with ^{99m}Tc EDDA 4 h after the Administration to a Mouse bearing Ehrlich Ascites Tumor (Anterior Projection)

An abscess was also imaged by ^{99m}Tc EDDA. A turpentine oil-liquid paraffin mixture (0.15 ml) was injected subcutaneously in the left foreleg of rats (female; 200±10 g body weight). Twenty four hours after the injection, the rats with an abscess were submitted to the scintigraphic study. The abscess was visualized 2 h after the administration of ^{99m}Tc EDDA. The abscess image was somewhat obscurred 4 h after the administration.

^{99m}Tc EDDA has been proved to be a new radiotracer that gives satisfactory scintigrams of Ehrlich ascites tumor. For scanning, ^{99m}Tc is of greater advantage than ^{67}Ga and ^{201}Tl for its good quality scans and the shorter interval between the administration and the scanning. However, many works have to be undertaken to find its place among other tumor specific agents which are in routine use. Further works are in progress by our hands and will be reported shortly.

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The Structures of Six Antifungal Oligoglycosides, Stichlorosides A_1, A_2, B_1, B_2, C_1, and C_2, from the Sea Cucumber *Stichopus Chloronotus* (BRANDT)

Chemical structures are reported for six antifungal lanostane-type triterpene-oligoglycosides from the Okinawan sea cucumber *Stichopus chloronotus* (BRANDT). The compounds are: stichlorosides A_1 (**9**), B_1 (**15**), C_1 (**20**) [having stichlorogenol (**3**) as the aglycone] and A_2 (**10**), B_2 (**16**), C_2 (**21**) [dehydrostichlorogenol (**4**) as the aglycone].

Keywords——sea cucumber; *Stichopus chloronotus*; stichloroside A_1; stichloroside A_2; stichloroside B_1; stichloroside B_2; stichloroside C_1; stichloroside C_2; lanost-7-ene type triterpene

In a recent communication,[1] we reported the isolation of six antifungal triterpene-oligoglycosides, stichlorosides A_1, A_2, B_1, B_2, C_1, and C_2, from the Okinawan sea cucumber *Stichopus chloronotus* (BRANDT) and clarified the structure of their aglycones named stichlorogenol (**3**) and dehydrostichlorogenol (**4**), which are respectively the common genuine aglycones of stichlorosides A_1, B_1, C_1, and A_2, B_2, C_2. This is a report on the structure of the parent oligoglycosides, stichlorosides A_1 (**9**), A_2 (**10**), B_1 (**15**), B_2 (**16**), C_1 (**20**), and C_2 (**21**).

Stichloroside A_1 (**9**)[1] is a hexaglycoside having two moles each of xylose (xyl), glucose (glu), and 3-O-methylglucose (3-Me-glu) in its oligosaccharide moiety.[2] The ^{13}C NMR spectrum of A_1 shows the β-glycosidic nature of these six monosaccharide moieties as judged by the anomeric carbon signals [δ_c 106.4, 105.5, 103.3, and 102.9 (all d)[3]] and also shows the presence of one acetoxyl group [δ_c 170.7(s)]. On acidic hydrolysis (2N aq. H_2SO_4), A_1 liberated two artifact aglycones **1**[1] and **2**,[1] showing that the oilgosaccharide moiety of A_1 attaches to 3β-OH and the acetyl group to 23-OH of stichlorogenol (**3**).

Alkaline treatment (1/6 N NaOMe–MeOH) of A_1 (**9**) gave desacetylstichloroside A_1 (**8**), $C_{66}H_{108}O_{32}\cdot 3H_2O$,[4] mp 211—212°C, $[\alpha]_D^{15}$ −36° (pyr.), which, on enzymic hydrolysis with crude naringinase,[5] yielded stichlorogenol (**3**) and three partial hydrolysates: A-pro-1 (**5**), $C_{35}H_{56}O_8$, mp 270—271°C, $[\alpha]_D^{21}$ −45° (pyr.), (monosaccharide composition[2]: xyl×1), A-pro-2 (**6**), $C_{48}H_{78}O_{18}\cdot 2H_2O$, mp 253—255°C, $[\alpha]_D^{18}$ −38° (pyr.), (xyl×1, glu×1, 3-Me-glu×1), and A-pro-3 (**7**), $C_{53}H_{86}O_{22}\cdot 2H_2O$, mp 256—257°C, $[\alpha]_D^{21}$ −44° (pyr.), (xyl×2, glu×1, 3-Me-glu×1).

Methylation of these hydrolysates with CH_3I-NaH–tetrahydrofuran[6] afforded their respective fully methylated derivatives: **5a** [anom. H at δ 4.21 (1H, d, J=7 Hz)], **6a** [δ 4.26 (2H, d, J=7), 4.63 (1H, d, J=8)], and **7a** [δ 4.24 (1H, d, J=8), 4.30 (1H, d, J=6), 4.62 (2H, d, J=6)]. Methanolysis of these methylated derivatives liberated the following methyl glycosides: Me 2,3,4-tri-O-Me-xylopyranoside from **5a**, Me 2,3,4,6-tetra-O-Me-glucopyranoside, Me 2,4,6-tri-O-Me-glucopyranoside, and Me 2,3-di-O-Me-xylopyranoside from **6a**, and Me 2,3,4-tri-O-Me-xylopyranoside, Me 2,3,4,6-tetra-O-Me-glucopyranoside, Me 2,4,6-tri-O-Me-glucopyranoside, and Me 3-O-Me-xylopyranoside from **7a**. Based on these findings, the structures of A-pro-1 (**5**), A-pro-2 (**6**), and A-pro-3 (**7**) have been substantiated.

Methylation[6] of desacetylstichloroside A_1 (**8**) gave an octadeca-O-methyl derivative (**8a**),