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1965

SovietRxiv

Abstract

Full Text

Chemistry

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On the Possibility of Using 3-(3,4-Dioxyphenyl)-alanine (DOPA) as a Carrier of Radioisotopes into Melanoma

One of the new methods for the selective action of ionizing radiation on the cells of tumor tissue is the saturation of a compound tropic to the given tumor with a therapeutic dose of a radioactive isotope of an element entering into it. Such an isotope may be tritium, whose sole β-radiation with $E_{\max} = 18$ keV and $E_{\text{av}} = 5$-6 keV and $T = 12.4$ years creates in tissue a localized region of ionization owing to the small range, not exceeding $1\,\mu$. In the organism tritium is not concentrated; its half-elimination period for humans is 9-14 days ([1]).

An essential condition for the use of a radioactive preparation for the purposes of diagnosis or radioisotopic therapy of a tumor is its selective action on tumor tissue. Obviously, this same requirement determines the successful use of other chemotherapeutic antitumor preparations.

The use of the peculiarities of the enzymatic apparatus of tumors is one of the possible ways of finding carriers of cytotoxic agents into a tumor. It is known that primary melanomas are insensitive to any of the existing chemotherapeutic preparations ([2]). On the basis of the distinctive character of the enzymatic complex of melanomas, the supposition was advanced that selective localization in them of pyrocatechin and tyrosine derivatives is possible ([3]). Among such tyrosine derivatives, which like it are readily attacked by the melanoma enzyme tyrosinase, is dioxyphenylalanine (DOPA), used in melanoma cells for the synthesis of the pigment melanin ([4]). In contrast, for example, to the amino acid tyrosine, DOPA is not used for the synthesis of tissue proteins. This permits one to think that, when introduced into the organism of an animal tumor-bearer, the preparation should selectively accumulate in the melanoprotein fraction of melanoma proteins and only in small concentration can be detected in other tissues. Its accumulation in this case, apart from melanoma, could be expected only in the sites of adrenaline synthesis, in particular in the adrenals, which raises the necessity of solving the question of a method for protecting the adrenals (and possibly the kidneys) from the action of the preparation.

The incorporation of DOPA into melanoma tissue has recently been studied by a number of authors ([5-9]). However, its distribution among the individual

protein fractions of melanoma has not yet been studied.

According to the Raper–Mason scheme, and also that of Duhon–Gregor, from DOPA formed from tyrosine under the influence of tyrosinase, two types of pigments may be synthesized: phenolic melanogens and indolic melanogens ([10,11]). This transformation proceeds according to Scheme 1.

Scheme 1

Tyrosine → DOPA, 3-(3,4-dihydroxyphenyl)alanine

DOPA → homovanillic acid → phenolic melanogens

DOPA → DOPA-quinone → leuco compound → DOPA-chrome → 5,6-dihydroxyindole → indole-5,6-quinone → indole melanogens

At the final stage of melanogenesis, melanogens of both types form complexes with protein and become part of melanoprotein.

The task of the present investigation was to clarify the question of the possibility of selective incorporation of DOPA into the melanoprotein fraction of melanoma proteins. For this purpose, 3-(3,4-dihydroxyphenyl)alanine, stably labeled with C^{14} in the second position of the side chain, was synthesized.

Experimental Part

According to the chosen scheme for the synthesis of labeled C^{14} 3-(3,4-dihydroxyphenyl)alanine, the starting product for the synthesis was glycine-$2C^{14}$ (see Scheme 2). The synthesis consists of five stages.

Scheme 2

Glycine-$2C^{14}$ → hippuric acid-$2C^{14}$ → 2-phenyl-4-(3,4-dimethoxybenzal)-5-oxazolone-$4C^{14}$ → N-benzoyl-3-(3,4-dimethoxyphenyl)aminoacrylic acid-$2C^{14}$ → N-benzoyl-3-(3,4-dimethoxyphenyl)alanine-$2C^{14}$ → 3-(3,4-dihydroxyphenyl)alanine-$2C^{14}$ (DOPA)

Reagents indicated in the scheme: C_6H_5COCl; veratric aldehyde; NaOH; sodium amalgam; HI, red phosphorus.

1. Condensation of glycine with benzoyl chloride to hippuric acid-$2C^{14}$ by the Schotten–Baumann reaction ([12]).
2. Preparation of 2-phenyl-4-(3,4-dimethoxybenzal)-5-oxazolone-$4C^{14}$ by fusion of hippuric acid with veratric aldehyde according to Erlenmeyer ([13]).
3. Hydrolysis of the azlactone with a 2% solution of caustic soda to N-benzoyl-3-(3,4-dimethoxyphenyl)aminoacrylic acid-$2C^{14}$ ([14]).
4. Reduction of the latter with 3% sodium amalgam to N-benzoyl-3-(3,4-dimethoxyphenyl)alanine-$2C^{14}$ ([14]).

Figure 1. Electropherograms of water-soluble melanoma proteins in agar-agar (A) and of the melanin-protein fraction (B)

Figure 1: Figure 1. Electropherograms of water-soluble melanoma proteins in agar-agar (A) and of the melanin-protein fraction (B)

5. Replacement of the methoxy groups of N-benzoyl-3-(3,4-dimethoxyphenyl)alanine by OH groups by boiling with freshly distilled hydroiodic acid (d = 1.7) in the presence of red phosphorus, with isolation of 3-(3,4-dihydroxyphenyl)alanine-2C (DOPA) ([15], [16]).

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Fig. 1. Electropherograms of water-soluble melanoma proteins in agar-agar (**A**) and of the melanin-protein fraction (**B**)

A special feature of the synthesis is strict control of the pH during isolation of DOPA from the acidic solution. The pH during neutralization of the acidic solution with 10% ammonia must not exceed 5.5, since the oxidation of DOPA is irreversible.

The chosen synthesis scheme made it possible to obtain DOPA-2C^{14} in 25% yield, with practically no loss of the specific activity of the starting product after five stages of synthesis of the preparation.

A specific feature of the product is its high oxidizability in air. The half-oxidation period of DOPA in water is 10 hr, as a result of which advance preparation of its aqueous solutions for biological experiments is not recommended.

The radiochromatographic purity of the product, determined in the methyl ethyl ketone–propionic acid–water system (75 : 25 : 30) in an atmosphere of hydrogen sulfide gas, was 98.5%. The specific activity of the isolated DOPA-2C^{14} was 1.4 mCi/g.

White mice with Harding–Passey melanoma were injected intraperitoneally with a solution of DOPA-2C^{14} at a dose of 4 μCi per animal. The mice were killed on the 2nd or 3rd day after administration of the preparation. The melanoma tissue was homogenized in phosphate buffer (pH 7.4; ionic strength μ 0.03) and extracted in the cold for 13–15 hr at 4°. The homogenate was centrifuged to separate tissue fragments at 18,000 rpm (15,000 g) and at 30,000 rpm (40,000 g) in an ultracentrifuge.

The protein content in the supernatant fluid was determined by the Lowry method. The water-soluble melanoma proteins were fractionated by electrophoresis in agar-agar gel at a current density of 12.5 mA/cm^2 in flowing veronal-acetate buffer (pH 8.6; μ 0.1) in the cold. After 12–13 hr of electrophoresis the agar-agar strips were cut out, dried, and stained with amido black (Amidoshwarz-10B).

The activity of the protein fractions in the electropherograms was measured

with a dismountable 4π counter registering 96–97% of all β-particles emerging onto the surface of the measured section of the electropherogram ([17], [18]). Electropherogram strips 3.5 cm wide were marked into sections 2 cm long. Thus the area of each measured section was 7 cm^2, and the surface in 4π geometry was 14 cm^2. The true activity of the protein fractions was determined by applying a self-absorption correction calculated for the 4π counter ([19]).

Results of the Study

As can be seen from the electropherogram (Fig. 1), the water-soluble melanoma proteins are clearly separated into 8 fractions (see also ([20], [21])). The melanin-protein fraction is distinctly visible on the unstained block (Fig. 1B). This fraction (5), moving slowly toward the anode, is located directly at the site of application of the protein under study. On the moist, undried block the band has the dark-brown color characteristic of melanin-protein. In addition, at the "start" (4) there remains a rather considerable amount, apparently, of denatured melanin-protein. After staining with amido black all fractions, including the melanin-protein fraction, are stained dark blue (Fig. 1A).

The intensity of incorporation of DOPA-$2C^{14}$ into the fraction of water-soluble melanoma proteins 43 hr after its introduction into the mouse organism is reflected in Table 1, which presents the results of one of many (15) experiments. In this experiment, of 37.9 mg of water-soluble melanoma proteins introduced into the agarose-gel well after electrophoresis, the greatest amount of protein (14.7 mg) was concentrated in fraction No. 5. This fraction included 67.5% of DOPA-$2C^{14}$; at the site corresponding to the application of protein (3.9 mg), 32.5% of DOPA-$2C^{14}$ remained. However, the specific

the activity of the proteins of fraction No. 4 proved to be almost twice as high as that of the proteins of fraction No. 5 ($4.73 \cdot 10^{-11}$ Cu/mg versus $2.61 \cdot 10^{-11}$ Cu/mg).

The presence of a high specific activity of the proteins of fraction No. 4, which contain melanoproteid and remained in the well at the site of application, may be explained by denaturation of part of the most labile proteins that had incorporated DOPA-$2C^{14}$, and by their retention at the site where the protein solution under study was applied. The radioactivity of all other protein fractions on the electropherogram did not exceed background level.

Table 1

Distribution of DOPA-$2C^{14}$ among fractions of water-soluble melanoma proteins

Fraction No.	Area of fraction, %	Amount of protein in fraction, mg	Protein activity of fractions (without background), imp/min	Protein activity of fractions (without background), dis/min	Protein activity of fractions (without background), in curies $×10^{10}$	Protein activity of fractions (without background), %	Specific activity of fraction protein, dis/min·mg	Specific activity of fraction protein, (curies/mg) $×10^{11}$
1	7.0	2.65	0	0	0	0	0	0
2	13.6	5.20	0	0	0	0	0	0
3	3.8	1.44	0	0	0	0	0	0
4	10.2	3.90	70	410	1.84	32.5	105	4.73
5	38.9	14.70	145	850	3.85	67.5	58	2.61
6	10.8	4.10	0	0	0	0	0	0
7	14.6	5.52	0	0	0	0	0	0
8	1.1	0.42	0	0	0	0	0	0
	100	37.93	215	1260	5.69	100	34	1.53

Note. 0—activity within background limits.

Thus, in the present work the selective incorporation of DOPA-$2C^{14}$ into the melanoproteid fraction of melanoma proteins has been demonstrated. This makes it possible to speak of the fundamental possibility of diagnosis and radioisotope therapy of melanomas by using DOPA as a carrier of radioisotopes into melanoma.

Work in this direction is continuing.

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Received
19 II 1965

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Note: Figure translations are in progress. See original paper for figures.