PHARMACOKINETICS OF [^{35}S]LEVAMISOLE

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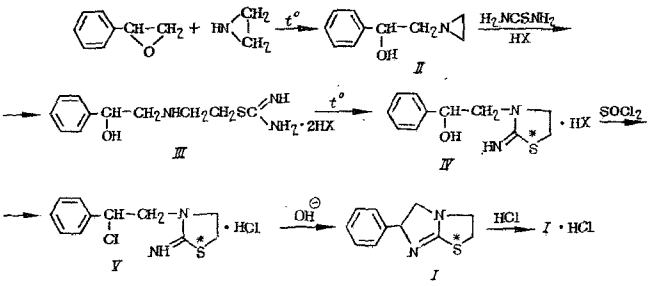
Levamisole (I) is used in the clinic as an agent promoting an enhancement of immune responses. According to the literature data [1-4], the preparation possesses the ability to stimulate the function of T-lymphocytes participating in reactions of immunity.

For a study of the behavior of (I) in the organisms, we synthesized ^{35}S-(I) and investigated its distribution in rats after intravenous injection. The preparation was synthesized according to the following scheme:



Styrene oxide and ethylenimine react, giving 1-(β-hydroxyphenyl)-ethylaziridine (IV). The aziridine (II) reacts with [^{35}S]thiourea in acid medium to give 3-(β-hydroxyphenylethyl)-3-iminothiazolidine (IV) through an isothiourea derivative (III). Replacement of hydroxybichlorine, followed by cyclization, catalyzed by a base, gives (I) with a specific mass activby of 35 mCi/g. In the case of intravenous injection of ^{35}S-I, a rapid decrease in the level of introduced radioactivity in the blood was observed. Although the coefficient of differential accumulation (CDA) of the label in the blood was 0.33 5 min after the injection, after 30 min the CDA was equal to 0.16. After 6 h the blood contained traces of radioactivity (CDA 0.016). The decrease in the label in the blood fitted into three exponential functions. The radioactivity corresponding to the CDA equal to 0.19 was removed from the blood with a half-life ($T_{1/2}$) of 10 min, while part of it, corresponding to CDA 0.082, had a half-life $T_{1/2}$ of 1 h. A small fraction of radioactivity (CDA 0.07) retained for the longest time can be distinguished: Its $T_{1/2}$ was equal to 27 h. In the first 15 min, as the radioactivity in the blood fell, there was an accumulation of it in the organs and tissues, with the exception of the pituitary, small and large intestines (Table 1).

The largest content of the label at CDA 1.04-4.6 was found in the following organs: liver (CDA 4.6), kidneys (CDA 3.06), pituitary (CDA 3.0), large intestine (CDA 1.58), adrenals (CDA 1.55), pancreas (CDA 1.21), small intestine (CDA 1.14), and lungs (CDA 1.04). A lower concentration of the preparation was detected in the thyroid gland (CDA 0.84), muscles (CDA 0.71), lymph nodes (CDA 0.62), spleen (CDA 0.46), stomach (CDA 0.43), brain (CDA 0.42), etc.

The label was removed from the organs and tissues over a period of 1-3 days. Its stay in the organism was 96-290 times as long as the period of accumulation (15 min). Although the extreme value of the label (CDA) in the period of its maximum content ranged from 0.3 (heart) to 4.6 (liver), in the period of the minimum value this index was from 0.001 (lymph nodes) to 0.23 (large intestine).

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TABLE 1. CDA of ^{35}S-I in Rats after Intravenous Injection

Tissue or organ	Time after injection of ^{35}S-I									
	5 min	15 min	30 min	1 h	2 h	3 h	6 h	24 h	48 h	72 h
Blood	0,33	0,17	0,16	0,10	0,09	0,06	0,05	0,04	0,02	0,01
Liver	1,36	4,60	1,44	1,42	0,63	0,36	0,23	0,15	0,10	0,06
Lungs	0,46	1,04	0,85	0,80	0,50	0,17	0,15	0,04	0,008	—
Kidneys	3,06	2,42	2,04	1,61	1,34	0,52	0,26	0,11	0,08	0,03
Spleen	0,46	0,25	0,20	0,14	0,12	0,10	0,08	0,07	0,008	—
Heart	0,30	0,23	0,22	0,18	0,10	0,09	0,05	0,03	—	—
Muscles	0,71	0,53	0,46	0,37	0,30	0,10	0,04	0,02	0,01	0,006
Skeleton	0,52	0,40	0,35	0,12	0,09	0,07	0,05	0,03	0,012	0,006
Thymus	0,10	0,40	0,33	0,30	0,21	0,07	0,02	0,01	—	—
Lymph nodes	0,32	0,62	0,55	0,42	0,34	0,15	0,03	0,004	0,002	0,001
Pancreas	0,25	1,21	1,0	0,81	0,76	0,30	0,12	0,06	0,03	0,02
Adrenals	0,36	1,6	1,18	1,04	0,94	0,37	0,03	0,02	—	—
Pituitary	0,60	2,41	3,0	1,27	1,15	0,50	—	—	—	—
Thyroid gland	0,60	0,84	0,70	0,65	0,62	0,43	0,26	—	—	—
Brain	0,08	0,42	0,32	0,20	0,12	0,05	0,03	—	—	—
Stomach	0,06	0,31	0,30	0,43	0,19	0,14	0,05	0,02	0,02	0,01
Small intestine	0,15	0,37	0,59	0,83	0,90	1,14	0,55	0,16	0,09	0,05
Large intestine	0,51	0,83	1,53	1,39	0,79	0,50	0,42	0,35	0,23	0,11
Tail (as site of injection)	2,68	1,53	0,96	0,73	0,82	0,73	0,38	0,20	0,07	0,04

In a comparison of the CDA of the radioactivity for tissues with its value for the blood it can be noted that in most of the organs it was greater than one. This permits us to consider that the preparation is found in the organs and tissues not only on account of its presence in the blood, but also thanks to its penetration into the structural formations of the tissues. Exceptions were the thymus, pancreas, brain, stomach, and small intestine, in which the ratio under consideration was less than one for 5 min after introduction of the preparation. This provides a basis for believing that in the organs noted the presence of the label was due primarily to its content in the blood. The highest ratio was in the kidneys (21), liver (14.2), small intestine (18), lungs (12), adrenals (10.4), pancreas (8.4), large intestine (9.3), and thyroid gland (8.0). In the remaining organs and tissues, the excess of the concentration of the label over its level in the blood ranged from 2.1 (spleen) to 4.3 (stomach). All this indirectly permits us to consider that the preparation and its metabolites are not simply contained in the blood, but are accumulated in the cells of the tissues.

The decrease in the total content of radioactivity in the organs of rats after intravenous injection of ^{35}S-I was rather rapid. After only 1 h, the organs and tissues contained 34.3% of the radioactivity introduced, and after one day 3.5%. On the basis of the exponential equation of the curve of the content of radioactivity, it can be concluded that the period of half-accumulation of the preparation was 10 min, while 40% of the label introduced was eliminated with $T_{1/2}$ equal to 10 min, 50% with $T_{1/2}$ equal to 23 h.

Estimating the content of ^{35}S-I in the organs and tissues according to the amount of the label at individual periods (Fig. 1), in this index we still do not consider the change in the concentration during the entire period of stay of the preparation in the organism. For this it was quite advisable to determine the integral content of the label. The index mentioned already reflects not only the amount, but also the corresponding time of the stay of the preparation and its metabolites. The ratio of the integral content of radioactivity in the organs of rats after intravenous injection of ^{35}S-I (CDA · h) during a 2-h observation period gives a somewhat different idea of the content of the label in comparison with the index CDA in individual periods. Of course, in the quantitative respect these two indices cannot be compared, since their estimation is based on different dimensions, but close values of the integral index in the blood, lymph nodes, stomach, and muscles can still be noted, while the CDA index in the blood is several times lower in comparison with that in the indicated organs. Substantial differences are also noted in other organs. The CDA·h index is especially high in the small and large intestines in comparison with the value in other organs, whereas the CDA index in the liver and kidneys is far higher than in the remaining organs. All this characterizes the large role of the time factor in the estimation of the content of the radioactive label.

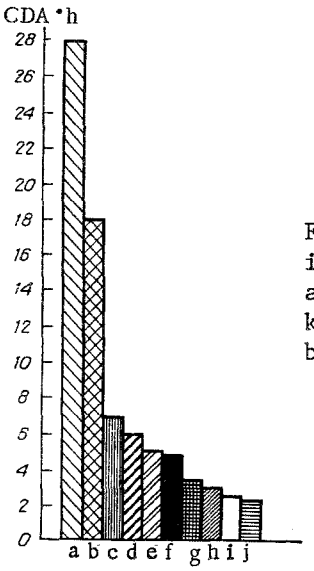

Fig. 1. Integral content (CDA·h) of ^{35}S-I in the organs and tissues of noninbred rats. a) Small intestine, b) large intestine, c) kidneys, d) liver, e) lungs, f) spleen, g) blood, h) lymph nodes, i) stomach, j) muscles.

Thus, our experimental investigations permitted us to establish that in the case of intravenous injection, ^{35}S-I is rapidly accumulated in the organs and tissues. Its largest concentration (CDA) is detected in the liver, kidneys, large and small intestines, and in the organs of the endocrine system. Considering that the concentration of ^{35}S-I is preserved in sufficient amounts for a period of up to 6 h (7% of the introduced radioactivity), the preparation should be administered no less than four times a day to maintain its content in the patient's body.

EXPERIMENTAL (CHEMICAL)

1-β-Hydroxyphenylethyl)aziridine (II). Styrene oxide (0.6 g, 0.005 mole) and ethylenimine (0.645 g, 0.015 mole) were heated in a heated ampule for 30 min at 100°C, the excess ethylenimine was distilled off under vacuum, and the light yellow oil remaining solidified upon standing. Recrystallization from cylohexane gave 0.58 g (71%) of the aziridine (II) [1] with mp 72-73°C.

[^{35}S](β-Hydroxyphenyl)-2-iminothiazolidine (IV). [^{35}S]Thiourea (0.3 g, 0.0039 mole, 100 mCi) and concentrated sulfuric acid (0.45 g, 0.0005 mole) were dissolved in water (4 ml), the solution obtained was cooled to 5°C, and a solution of (II) (0.58 g, 0.0036 mole) in dioxane with a volume of 1 ml was added to it dropwise with mixing for 10 min. The solution obtained was condensed under vacuum and then boiled at 100°C for 3 h with a reflux condenser. After cooling, chloroform (3.5 ml) and a cold (5°C) solution of 20% NaOH were added to pH 11.0-12.0. The chloroform layer was removed, and then the aqueous layer was washed once again with chloroform, the extracts were dried over Na_2SO_4 and the ammonia dissolved was purged with dry air. It was treated with a saturated solution of HCl in ethanol to pH 2.0. After 2 h exposure at 2°C the product was filtered off, washed with a chloroform–ethanol mixture (4:1), and dried. Yield of (III) 0.66 g (82%).

Hydrochloride of ^{35}S-I. A solution of 3-(β-hydroxyphenylethyl)-2-iminothiazolidine (0.66 g) in 3.5 ml methylene chloride was heated to 40°C, and thionyl chloride (0.22 ml, 50 min) was added. Then the mixture was cooled and 1.5 ml of water and 1.5 g $NaHCO_3$ were added. The mixture obtained was again heated for 2 h at 60°C. After the end of the reaction the solvent was removed from the cooled reaction mass, dried over Na_2CO_3, and filtered. Then gaseous HCl was passed through the solution, and it was boiled to remove excess HCl. The solution was cooled at 0°C, and ^{35}S-I hydrochloride was obtained, yield: 0.224 g, or 36.5%, mp 260°C, specific mass activity 35 mCi/g. The identification and estimation of the radiochemical purity of the compound obtained were performed by the method of thin-layer chromatography on Silufol plates. The solvent system benzene–ethanol (3:2) was used as the mobile phase. One spot, coinciding in R_f value with samples of the pharmacopoeia preparation (R_f 0.3), corresponded to the pure preparation.

EXPERIMENTAL (BIOLOGICAL)

Radiometry of Internal Organs. The experiments were conducted on 40 noninbred white rats. The preparation, in the form of an aqueous solution, was injected into the tail vein. The radioactivity of the injected preparation was 5 μCi per rat. At various periods after the injection of ^{35}S-I the rats were decapitated. Samples of the organs and tissues were hydrolyzed in 1 N solution of alkali at 80-90°C. The hydrolysate obtained was neutralized with glacial acetic acid to pH 7.0-8.0. Radiometry was conducted on an Anistron scintillation counter. The results were evaluated according to two indices: according to CDA, characterizing the ratio of the concentration of the recorded radioactivity in counts in the tissues to the injected radioactivity in counts per gram of weight of the rat, and according to the percent accumulation of the label in the organs and tissues. The elimination of radioactivity from the bodies of the rats was estimated according to an exponential equation.

$$A_t = a_1 \cdot e^{-\lambda_1 \cdot t} + a_2 \cdot e^{-\lambda \cdot t},$$

where A_t is the concentration or total content of radioactivity in the time t, a_1, amount of radioactivity in the time t_0 of the first exponent, a_2, amount of radioactivity in the time t_0 of the second exponent, λ_1 and λ_2, indices of the constant decrease in radioactivity, corresponding to the first and second exponents, t, time, hours. On the basis of the exponential relationship established between the content of radioactivity in the tissues and the time elapsed after injection of the preparation, the integral content of the label was judged. The latter corresponds graphically to the area representing the sum of infinitely small areas bounded by the curve characterizing the concentration of radioactivity (plotted along the y axis) as a function of the time (plotted along the x axis). Then

$$\sum_{n \to \infty} a_t = \int_0^{\infty} A_t \cdot e^{-\lambda \cdot t},$$

where Σa_t is the sum of the radioactivity, expressed in CDA·h and corresponding to the area S, n, number of intervals between the points along the x axis; $\int_0^{\infty}$, the integral characterrizing the change in the amount of radioactivity (in our case expressed in terms of CDA) from zero to infinity, A_t, maximum amount of radioactivity in the time t; e, base of natural logarithms, t, time, hours, a_t, the gain in time. After integration of this mathematical expression, it follows that area $S = A_t(T_{1/2}/0.693)$, where $S = \lim \Sigma a_t$ when $n \to \infty$. On the basis of the expression obtained we calculated the areas bounded by the indices under consideration. The period of half-accumulation of the label was estimated indirectly, for this the readings of the exponent situated from the zero ordinate up to the time of the end of accumulation were deducted from the indices of accumulation. The data obtained were outlined in the form of an exponent (with opposite sign), which corresponded to the accumulation.

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