ON THE METABOLIC DETOXICATION OF 4-IODOTHYMOL IN MAN

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Abstract.....The detoxication of 4-iodothymol was investigated in man.

A new metabolite was isolated as a crystalline derivative from 4-iodothymol medicated urine and identified as 4-iodothymol glucuronide as a methyl acetyl derivative.

The deiodinated metabolite of 4-iodothymol, thymoquinone, was also recognized in a very small amount in 4-iodothymol medicated urine.

Key Words : 4-Iodothymol, Parasiticide, Glucuronic Acid Conjugation, Deiodination, Thymoquinone.

4-Iodothymol, 6-iodo-3-hydroxy-1-methyl-4-isopropyl benzene, is an iodine derivative of thymol, but as the result of iodination of thymol, the toxicity of 4-iodothymol is considerably reduced as compared with that of thymol. 4-Iodothymol was originally developed as an anthelminic for animals, but recently this compound has been clinically used as a parasiticide for various gastrointestinal parasites.

In the course of investigations involving the chemotherapy of alveolar echinococcosis of liver in man, 4-iodothymol was introduced for testing the treatment of the helminthiasis based on laboratory trials of a series of thymol derivatives.

It attracted our attention to investigate the metabolic fate of 4-iodothymol, as no report dealing with the metabolic fate of 4-iodothymol has been published.

In the present paper, the metabolic fate of 4-iodothymol in man was studied, especially concerning the extent of its conjugation, the isolation of its main metabolites, and the possibility of its deiodination.

METHODS

Extraction and fractionation of metabolites of 4-iodothymol (from [M-1] to [M-4]) were performed by the procedure shown in Chart. 1.

Preparation of Standard Substances

4-Iodothymol : 4-iodothymol was synthesized by the usual method (Sister Miriam Grace Solomon 1964). mp 66-67°C.

Thymoquinone : Thymoquinone was synthesized by the usual method (H. Gilman, 1963).

Chart 1. Extraction and Fractionation Procedures of Metabolites

Isolation of 4-Iodothymol glucuronide from Human Urine (Aoki, 1962., Kamil et al., 1951)

Four grams of 4-iodothymol were orally given to a volunteer and 24 hour urine specimens (total 1.65 L) were collected and filtered. The filtrate was adjusted to PH 6 with 3N-NaOH, and continuously extracted with Et₂O for 24 hours to eliminate Et₂O soluble substances. Then the urine was adjusted to PH 3 with 3N-HCl and was continuously extracted with Et₂O for 60 hours. The Et₂O extract was evaporated to dryness under reduced pressure. The residues were dissolved in 100 ml of Et₂O-MeOH (9:1) mixed

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solvent and poured into 500 ml of petroleum ether (bp. 40–60°C) to precipitate glucuronide. The obtained red-brown precipitates were dissolved in 100 ml of MeOH and 30 ml of CH_2N_2 - Et_2O solution was added to the MeOH solution. The solvent was removed by evaporation under reduced pressure and the residues were dissolved in 10 ml of pyridine and 10 ml of Ac_2O . After standing at room temperature for two days, the reaction mixture was poured into 500 ml of ice water. The crude brown precipitates were collected by filtration and dissolved in a small amount of EtOH. After treatment with active charcoal and several successive recrystallization from EtOH, fine white needles were obtained. mp 145–147°C.

Yield 0.28 g, $[\alpha]_D^{20} = -25.0^\circ \text{C}$ ($C=1$ in CHCl_3).

IR : $\nu_{\text{max}}^{\text{nujol}}$ cm^{-1} : 1240, 1762 (CO).

Anal. Calcd. for $\text{C}_{23} \text{H}_{29} \text{O}_{10} \text{I}$: C, 46.63 : H, 4.89 : Found : C, 46.59 : H, 4.94.

Analytical Method

Glucuronic acid : Total glucuronic acid was measured by the naphthoresorcinol picrate method modified by Ishidate et. al. (Ishidate and Nambara 1957).

Ethereal sulfuric acid : This was determined by Fiske's modification of benzidine method.

Gas Chromatography

The instrument used for this work was Shimazu Model GCIB Type gas chromatograph equipped with a hydrogen flame ionization detector. The column packing was 1.5% SE-30 on chromosorb. The column temperature was maintained at 150°C and detector cell temperature was 200°C. The flow rate of N_2 was 35 ml/min, H_2 was 80 ml/min, and air was 0.8 liters/min.

Enzymatic Hydrolysis of 4-Iodothymol Medicated Urine (Nakado et. al, 1954)

4-Iodothymol medicated urine (4 g/body, 24 hours urine) was adjusted to pH 6 with 3 N-NaOH and continuously extracted with Et_2O for 24 hours. Then the urine was adjusted to PH 2 with 3 N-HCl and extracted with Et_2O for 60 hours. The PH 2 Et_2O extract was evaporated to dryness under reduced pressure. The residues were dissolved in a mixture of 100 ml of Et_2O -MeOH (9 : 1), poured into 500 ml of petroleum ether (bp. 40–60°C), and restored in a refrigerator overnight. The brown precipitates obtained were dried, dissolved in 200 ml of H_2O , and adjusted to PH 5 with 0.1N-NaOH. To 20 ml of the solution, 15 ml of 0.1 M-acetate buffer (PH 5.0) and 19500 Fishman units of β -glucuronidase (Tokyo Zoki) were added, and incubated at 37°C for 24 hours. After incubation, the reaction mixture was extracted with 20 ml of ethylene dichloride, and the extract was evaporated to dryness. The residues were dissolved in a small amount of EtOH and applied to gas chromatography.

RESULTS AND DISCUSSION

Table 1 shows a 48 hour output of glucuronic acid and ethereal sulfuric acid in the urine of two volunteers. As seen in the table, excretion of glucuronic acid and ethereal sulfuric acid after the administration of 4-iodothymol has markedly increased over 24

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Table 1. Glucuronic Acid and Ethereal Sulfate excreted in Human Urine 48 hours After the Administration of 4-Iodothymol

		Urine			
		Control	Control	24 hr	48 hr
Glucuronic acid	A	455.4	568.8	2177.7	812.8
	B	537.2	541.2	2329.2	742.5
Ethereal sulfate	A	87.2	70.0	219.6	133.3
	B	247.1	126.7	492.8	252.0

Results are expressed in mg excreted for every 24 hours. A and B indicate individuals who took 4g of 4-Iodothymol orally.

hours. The amount of the increased excretion of glucuronic acid was prevalent for 48 hours after the administration. The increased excretion of glucuronic acid and ethereal sulfuric acid suggested the possibility of a glucuronic acid conjugation and an ethereal sulfuric acid conjugation which is derived from 4-iodothymol or any biotransformed product of 4-iodothymol.

Isolation of the glucuronic acid conjugate of 4-iodothymol was undertaken by the usual method. Methylation and acetylation of the glucuronide fraction resulted in the isolation of a fine crystalline substance. By several qualitative and quantitative evidences, this isolated substance was identified as a methyl acetyl derivative of 4-iodothymol glucuronide.

It should be remembered that many of the anthelmintic drugs are toxic, and consecutive administration of the drugs at a regular interval is likely to cause several undesirable side effects. Therefore, the adequate metabolic detoxication of an anthelmintic is needed in order to avoid the undesirable side effects due to the accumulation of the drug in the body. From this study, 4-iodothymol was ascertained to be metabolized in considerable extent to form glucuronide and possibly ethereal sulfate in man.

Several attempts to synthesize this methyl acetyl derivative of 4-iodothymol glucuronide ended in failure, because in the initial stage of the synthetic reaction, 4-iodothymol was decomposed with iodine liberation under experimental conditions (Bollenback et. al, 1955).

In order to elucidate the more detailed biotransformations of 4-iodothymol, gas chromatographic identification of each urinary fraction which was fractionated by Chart 1, was undertaken, and the main results are shown in Fig. 1.

The M-1 fraction contained 4-iodothymol and thymoquinone, but the M-4 fraction contained only 4-iodothymol. From this fact, it is evident that a very small amount of thymoquinone is excreted into 4-iodothymol medicated urine in an unconjugated form. Probably, a small portion of the medicated 4-iodothymol is deiodinated to form thymoquinone in vivo. The deiodination of 4-iodothymol in vivo is very interesting in its correlation with the metabolism of thymol, which has been reported with the isolation of thymol glucuronide (as trichloro-derivative) and the detection of thymohydroquinone in urine (Katsuyama 1898, Takao 1923, Robbins 1934.).

It is also very interesting that, in spite of marked reactivity of iodine in 4-iodothymol

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Fig. 1. Gas Chromatogram of the Urinary Extracts
Chart-1 : M-1 Fraction
Chart-2 : M-4 Fraction
1, thymoquinone ; 2, 4-iodothymol.

molecule as exhibited in the forementioned, the 4-iodothymol glucuronide synthetic reaction, iodine in 4-iodothymol is quite stable in vivo and only a very small portion of 4-iodothymol is deiodinated to form thymoquinone.

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