ON THE METABOLIC DETOXICATION OF BITHIONOL IN MAN

Masahiko TAKADA

Hokkaido Medical Affairs Bureau, Ministry of Health and Welfare, Sapporo*

Received November 15, 1975.

Summary—Detoxication of bis (2-hydroxy-3, 5-dichlorophenyl) sulfide, bithionol, was investigated in man. A novel metabolite was isolated as a crystalline derivative from bithionol medicated urine and identified as bithionol-monoglucuronide (as a methyl acetyl derivative). Oxidation of the sulfur atom of this compound was not recognized. It is concluded that bithionol is detoxicated partially by forming a monoglucuronide conjugate.

Bithionol, bis (2-hydroxy-3, 5-dichlorophenyl) sulfide, has been widely used as a skin disinfectant and as an additive of soaps and cosmetics. Recently, clinical usefulness of bithionol for the treatment of paragonimiasis in man has been reported (Yokogawa et. al. 1961). Bithionol is administered orally in a dose of 2 g per day usually for a period of ten days for the treatment of paragonimiasis in man. As a result of continuous administration of the drug, several undesirable side effects also have been reported (Yokogawa et. al. 1961).

In order to elucidate the actual feature of toxicity in this drug, its metabolic fate in man should be taken under consideration.

The present investigation was undertaken to identify and isolate the main metabolites of bithionol in man.

Methods

Preparation of Standard Substances

Bithionol: Commercially available bithionol was purified by recrystallization from Me_2CO mp 184~185.5°C.

Bis (2-hydroxy-3, 5-dichlorophenyl) sulfoxide: Commercially available bis (2-hydroxy-3, 5-dichlorophenyl) sulfoxide was purified by recrystallization from iso-PrOH. mp 206°C.

Bis (2-hydroxy-3, 5-dichlorophenyl) sulfone: This substance was synthesized by the usual method (Mauthner 1906, Gump and Vitucci 1945). mp 168~169°C.

Bithionol-glucuronide (Bollenback et. al. 1955): To 14 g of bithionol 4 g of methyl (tri-*o*-acetyl- α -D-glucopyranosyl bromide) uronate and 4 g of AgCO_3 were added and dissolved in 150 ml of acetonitrile. The mixture was stirred for 48 hours at room temperature continuously, and filtered to eliminate silver-black precipitates, which were washed repeatedly with acetonitrile. The filtrate and the washings were combined and evaporated to dryness under reduced pressure at 40°C. The resulting white-brown scaly residues were dissolved in 100 ml of CHCl_3 and filtered. The CHCl_3 solution was then washed repeatedly with 0.5 N-KOH and H_2O , dried with anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The residues were dissolved in a small amount of hot EtOH and treated with active charcoal. White leaflets were obtained by several recrystallization from EtOH. Yield 0.9 g, mp 198~200°C.

The IR absorption spectra of this substance is illustrated in Fig. 3.

Anal. for $\text{C}_{25}\text{H}_{22}\text{O}_{11}\text{SCl}_4$, Calcd.: C, 44.64; H, 3.27

Found.: C, 44.71; H, 3.34

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

* Location : Nishi-18-chome, Minami 1-jo, Chuoku, Sapporo

Detoxication of Bithionol

Two grams of bithionol were orally given to two volunteers and 24 hours urine specimens (total 3.1 L) were collected and filtered. The filtrate was adjusted to PH 3 with 3 N-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 50 ml of H₂O. The solution was filtered and the filtrate was again evaporated to dryness under reduced pressure.

The residues were dissolved in a mixture of 50 ml of Et₂O and 20 ml of MeOH, and poured into 500 ml of petroleum ether (bp 40~60°C) to precipitate glucuronide. The brown syrup which was precipitated from the petroleum ether was dried. The syrup was dissolved in 30 ml of MeOH, and 30 ml of CH₂N₂-Et₂O solution was added to the MeOH solution. The reaction mixture was restored in a refrigerator for three days. The solvent was removed by evaporation under reduced pressure and the residues were dissolved in 10 ml of pyridine and 10 ml of Ac₂O. 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, white fine leaflets were obtained.

Yield 0.03 g, mp 198~200°C.

Anal. for C₂₅H₂₂O₁₁SCl₄, Calcd.: C, 44.64; H, 3.27.

Found.: C, 44.63; H, 3.37.

The IR absorption spectra of this substance is illustrated in Fig. 3.

Thin-Layer Chromatography

The samples were applied to Kieselgel GF plates (adsorbents were activated at 110°C for one hour, 0.5 mm in thickness), and were developed with solvent systems of (1) PrOH-NH₄OH(7:3), (2) PrOH-H₂O-NH₄OH(6:1:2), (3) iso-PrOH-NH₄OH-H₂O(20:1:2), (4) Et₂O-benzene(1:1), (5) Et₂O-petroleum ether (1:1) and (6) CHCl₃-Et₂O(3:1). The plates were dried, checked by illumination from ultraviolet rays, and sprayed with the following detecting reagents: (1) naphthoresorcinol picrate reagent: 1% naphthoresorcinol picrate in EtOH to which an equal volume of 20% H₂SO₄ was added before spraying. The sprayed plate was heated at 110°C for one hour. (2) potassium ferricyanide-ferric chloride reagent: mixed solution of the same volume of 1% potassium ferricyanide solution and 1% ferric chloride solution. (3) TCNE reagent: 2% tetracyanoethylene in benzene. (4) chloranil reagent: 1% tetrachloro-p-benzoquinone in benzene. (5) Gibbs reagent: 2% 2,6-dibromo-N-chloro-p-benzoquinoneimine in benzene.

The plates were sprayed first with one of (3)~(5) chromogenic reagents and then exposed to ammonia vapors (Fishbein and Fawkes 1966).

Enzymatic Hydrolysis of Bithionol-glucuronide in Urine (Nakado et. al. 1954)

Two grams of bithionol were orally given to a volunteer and a 24 hours urine specimen was collected. The total urine was adjusted to PH 6 and continuously extracted with Et₂O for 24 hours. Then the urine was adjusted to PH 3 and extracted with Et₂O continuously for 60 hours. The PH 3-Et₂O extract was evaporated to dryness under reduced pressure. The residues were dissolved in a mixture of 50 ml of Et₂O and 20 ml of MeOH, 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 H₂O, and adjusted to PH 5 with 0.1 N-NaOH. To 20 ml of the solution, 15 ml of 0.1 M-acetate buffer (PH 5.0) and 19,500 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 chloride, and the extract was evaporated to dryness. The residues were dissolved in a small amount of Et₂O and applied to thin-layer chromatography.

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.

Results and Discussion

Table 1 shows 24 hours outputs of glucuronic acid and ethereal sulfate in the urine of two volunteers. As seen in the table, glucuronic acid excretion was increased after administration of bithionol, while ethereal sulfate excretion was markedly decreased.

The increased excretion of glucuronic acid suggested the possibility of glucuronic acid conjugation,

Table 1. Glucuronic Acid and Ethereal Sulfate excreted in Human Urine
24 hours after Administration of Bithionol

	Volunteer	Urine	
		Control	24 hr
Glucuronic acid	A	466.0	609.5
	B	410.7	579.8
Ethereal sulfate	A	443.8	47.3
	B	289.0	26.6

Results are expressed mg excreted for 24 hours. A and B indicate individuals who took 2g of bithionol orally.

Fig. 1 Thin-Layer Chromatograms of Bithionol, Its Expected Metabolites, and the Urinary Extracts with Various Solvent Systems.

Solvent Systems.

A : PrOH-NH₄OH (7 : 3)

B : PrOH-NH₄OH-H₂O (6 : 2 : 1)

C : iso-PrOH-NH₄OH-H₂O (20 : 1 : 2)

Standard Substances and the Urinary Extracts.

BT : Bithionol

BT-SO : Bis (2-hydroxy-3, 5-dichlorophenyl) sulfoxide.

BT-SO₂ : Bis (2-hydroxy-3, 5-dichlorophenyl) sulfone.

UE-1 : Bithionol dosed urine-PH 6 Et₂O extract.

UE-2 : Bithionol dosed urine-PH 3 Et₂O extract.

UE-3 : Bithionol dosed urine-PH 3 Et₂O extract

β -glucuronidase hydrolysate-ethylene dichloride extract.

Detoxication of Bithionol

which derived from bithionol or any biotransformed product of bithionol. As reduced excretion of ethereal sulfate is concerned, no explanation is available.

The 24 hours urine specimens of two volunteers who were orally given 2g of bithionol were adjusted to PH 6, and were continuously extracted with Et₂O for 24 hours (UE-1). Then the urines was adjusted to PH 3 and extracted with Et₂O for 60 hours (UE-2). A portion of UE-2 was evaporated

Fig. 2 Thin-layer Chromatograms with Various Solvent Systems
Solvent Systems

(A) Et₂O-benzene (1 : 1)

(B) Et₂O-petroleum ether (1 : 1)

(C) CHCl₃-Et₂O (3 : 1)

BT-GA(B) : Biosynthetic bithionol-glucuronide
(as methyl tetra-acetate)

BT-GA(C) : Chemically synthetic bithionol-glucuronide
(as methyl tetra-acetate)

Fig. 3 IR Absorption Spectra of Bithionol-glucuronide

A : biological sample

B : authentic sample

Both samples are methyl-tri-acetyl derivative.

Fig. 4 Formation of Bithionol Glucuronide

to dryness and the residue was incubated with β -glucuronidase. The reaction mixture was then extracted with ethylene dichloride (UE-3). UE-1, UE-2 and UE-3 were all applied to thin-layer chromatography with reference substances which were expected as bithionol metabolites. As shown in Fig. 1, although no trace of unchanged bithionol was identified in UE-1 and UE-2, UE-3 contained a considerable amount of bithionol. However, no structurally modified product of bithionol was detected chromatographically in the three extracts.

From the results mentioned above, it was speculated that bithionol forms a conjugate with glucuronic acid in man. Thus, the following experiments were carried out to isolate and to identify the glucuronic acid conjugate. After oral administration of bithionol to two volunteers in a dose of 2 g, a 24 hours urine specimen was collected. Total urine was extracted with Et_2O at pH 3 for 60 hours. The Et_2O extract was treated to obtain methylated and acetylated gum, which was purified by recrystallization from EtOH . The purified substance was identified with the chemically synthesized bithionol-glucuronide by several qualitative and quantitative examinations. Thin-layer chromatographic behavior of bithionol glucuronide (as a methyl acetyl derivative) is also shown in Fig. 2.

From the data of the infrared absorption spectrum (Fig. 3) and elemental analysis, the obtained glucuronide was confirmed to be monoglucuronide of bithionol, in which one of two phenolic hydroxyl groups in the bithionol molecule is intact. This fact suggests that one of the phenolic hydroxyl groups of bithionol sustains somewhat steric hindrance. The conjugation with glucuronic acid with one of hydroxyl groups of bithionol may hinder another hydroxyl group in forming a conjugate with glucuronic acid.

Primarily, it was speculated that the sulfur atom in the bithionol molecule would be changed in vivo to sulfoxide or sulfone, but no such biotransformed products were identified by thin-layer chromatography in the urine of bithionol medicated volunteers as shown in Fig. 1.

It is generally believed that the possible metabolic alterations of aryl sulfides are the oxidation of the sulfur atom to sulfoxide or sulfone, or glucuronide formation following hydroxylation of the aryl groups (Clare 1940, Walkenstein and Seifter 1959, Salzman and Brodie 1956, Burns et al 1957, Rose and Spinks 1948, Maw 1953).

Bithionol is detoxicated by forming glucuronide, but the sulfur atom of this compound is not oxidized to form sulfoxide or sulfone in the way other aryl sulfides are.

Acknowledgement

The author expresses his deep gratitude to Dr. T. Kosaka, Director of the Hokkaido Medical Affairs Bureau, for his encouragement and permission for publication of this work, and also to Dr. T. Tanabe, Professor of Higashi-Nippon Gakuen University, for his kindness in the correction and publication of this manuscript. Thanks are also given to Dr. M. Haga, Professor of Higashi-Nippon Gakuen University, for the useful discussion and technical assistance during this work.

References

- AOKI, I. (1962). Chem. Pharm. Bull. Tokyo, 10, 105.
- BOLLENBACK, G. N., LONG, J. W., BENJAMIN, D. G. & LINDQUIST, J. A. (1955). J. Amer. Chem. Soc., 77, 3310.
- BURNS, J.J., YÜ, T.F., RITTERBAND, A., PEREL, J.M., GUTMAN, A.B. & BRODIE, B.B. (1957). J. Pharmacol. Exptl Therap., 119, 418.
- CLARE, N. T. (1940). Aust. Vet. J., 23, 340.
- FISHBEIN L. & FAWKES, J. (1966). J. Chromatography, 22, 323.
- GUMP, W.S. & VITUCCI, J.C. (1945). J. Amer. Chem. Soc., 67, 238.
- ISHIDATE, M. & NAMBARA, T. (1957). Chem. Pharm. Bull. Tokyo, 5, 515.
- KAMIL, I.A., SMITH, J.N. & WILLIAMS, R.T. (1951). Biochem.J., 50, 235.
- MAUTHNER, F. (1906). Chem. Ber., 39, 1351.
- MAW, G.A. (1953). Biochem. J., 55, 42.
- NAKADO, T., AIZAWA, Y. & IMAI, E (1954). Endocrinol. Japon., 1, 196.
- ROSE, F.L. & SPINKS, A. (1948). Biochem. J., 43, vii.
- SALZMAN, N.P. & BRODIE, B.B. (1956). J. Pharmacol. Exptl. Therap., 118, 46.
- WALKENSTEIN, S.S. & SEIFTER, J. (1959). J. Pharmacol. Exptl. Therap., 125, 283.
- YOKOGAWA, M. et. al. (1961). Chiriyo, 43, 917.
- YOKOGAWA, M. et. al. (1961). Chiba Igakukai Zasshi, 37, 16.