Acta Medica Okayama

Volume 18, Issue 2

1964 April 1964 Article 2

Experimental isovalthinuria IV. Incorporation of S35-Methionine or S35-Cystine into urinary isovalthine

Toshihiko Ubuka*Katsumi Horiuchi†Takehira Shimomura‡Shunzi Mizuhara**

*Okayama University, [†]Okayama University, [‡]Okayama University, **Okayama University,

Copyright ©1999 OKAYAMA UNIVERSITY MEDICAL SCHOOL. All rights reserved.

Experimental isovalthinuria IV. Incorporation of S35-Methionine or S35-Cystine into urinary isovalthine*

Toshihiko Ubuka, Katsumi Horiuchi, Takehira Shimomura, and Shunzi Mizuhara

Abstract

In the course of experimental isovalthinuria induced by cholic acid, S35-methionine or S35cystine administered was incorporated into urinary isovalthine in guinea pigs. Sulfur atom of cysteine seems to be utilized much better for isovalthine synthesis than that of methionine.

*PMID: 14204460 [PubMed - indexed for MEDLINE] Copyright ©OKAYAMA UNIVERSITY MEDICAL SCHOOL

Acta Med. Okayama 18, 65-70 (1964)

EXPERIMENTAL ISOVALTHINURIA

IV. INCORPORATION OF S³⁵-METHIONINE OR S³⁵-CYSTINE INTO URINARY ISOVALTHINE

Toshihiko UBUKA, Katsumi HORIUCHI, Takehira SHIMOMURA, and Shunzi MIZUHARA

Department of Biochemistry, Okayama University Medical School, Okayama Japan

Received for publication, March 12, 1964

In previous paper on experimental isovalthinuria¹, a following fact was reported. When guinea pigs were fed on bile acids or hypocholesterolemic agents, a maximum peak of isovalthine excretion was observed at around the fourth week after the start of administration and after that isovalthine disappeared from urine even when the feeding on these inducers kept continued. When methionine was administered at such time, remarkable isovalthinuria was again observed.

Taking advantage of the above phenomenon, the utilization of sulfur atom of methionine or the isovalthine synthesis has now been proven.

MATERIALS AND METHODS

Six male guinea pigs weighing 370-420 g are divided into three groups and each group is placed in one cage. Basal diet, conditoning of room temperature, and urine collection are the same as described in a previous paper¹.

Five mg of cholic acid per animal per day are given to all groups as the isovalthinuria inducer for six weeks. On the sixth week only, 5 mg of L-cysteine per animal per day are given to a control group for six days and S³⁶-amino acids are given to the experimental groups as follows. Commercial S³⁵-methionine (2.29 mc) or S³⁶-DL-cysteine (2.34 mc) is mixed with each 40 mg of non-labeled L-methionine or L-cysteine respectively, and each mixture is dissolved in 4 ml of water and 0.5 ml of the solution per animal per day is orally administered for 4 days. In the last two days on the sixth week, 5 mg of each non-labeled sulfur amino acid per animal per day are given.

Acidic amino acid fraction of weekly urine of each group is analyzed on an amino acid analyzer (Beckman Model 120 B) as described previously¹.

In the cases of S⁸⁵-containing acidic amino acid fraction of the sixth week, the total fraction is treated on a preparative column $(1.8 \times 150 \text{ cm})$ of the analyzer. One tenth of the effluent is removed for ninhydrin analysis by using stream

T. UBUKA, K. HORIUCHI, T. SHIMOMURA, and S. MIZUHARA

divider accessory and the remainder portion of the effluent is diverted to a fraction collector which collects every 5 ml in each tube.

When a radioactive sample is dried in a stainless planchet for gas-flow counter, one drop of 0.5 % alkylbenzene sulfonate is added in all cases.

RESULTS

1. Control Group

66

The total amount of urinary isovalthine excreted by two control guinea pigs in each was as follows: up to the end of the 3rd week, 0; 4th week, 0.65 μ mole; 5th week, 7.12 μ moles; 6th week (cysteine administered), 9.52 μ .moles. Thus the administration of cysteine instead of methionine appears also to enhance isovalthine excretion.

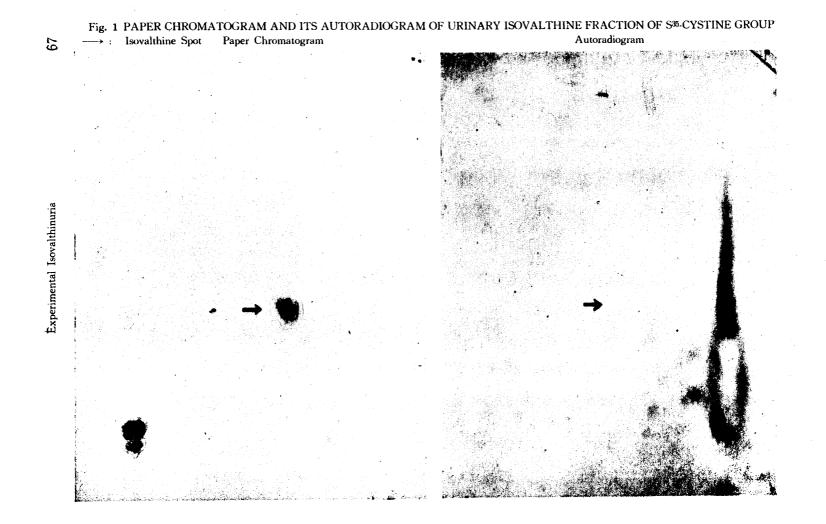
2. S³⁵ - Cystine Group

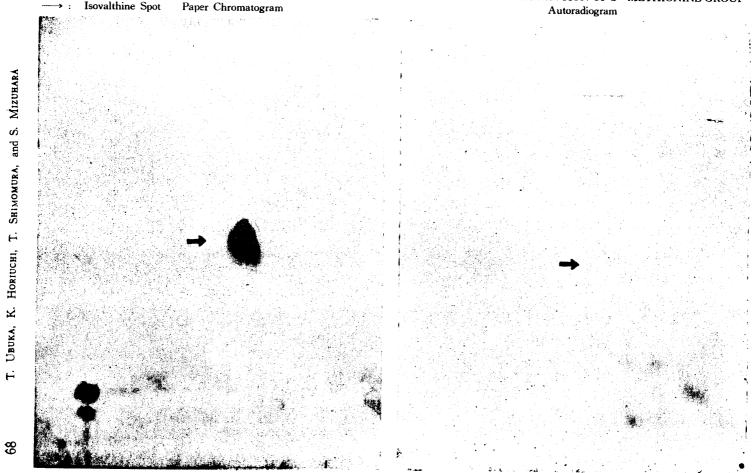
The total amount of urine excreted on the 6th week was 600 ml. The amount of isovalthine in the acidic acid fraction was calculated to be 11.9 μ moles (2.63 mg).

The isovalthine containing fractions collected in tubes of fraction collector were mixed and deionized once with a column containing strong cation exchanger (H-form). The ammonia eluant from the column was dried in vacuum and transferred into a planchet with a small amount of water. The dried matter in the planchet was 29.17 mg which should contain 2.39 mg $(2.63 \times 9/10)$ of isoval-thine. The sample showed 36, 627 cpm.

The dried matter in the planchet was dissolved in 0.1 ml of water and 0.02 ml of the solution was developed one dimensionally on a paper strip by high voltage electrophoresis². The electropherogram was colored with ninhydrin and tested in a paper chromatogram scanner. On a scanner chart, however, starting line showed the highest radioactivity and isovalthine band showed an indistinct peak. The isovalthine band of the electropherogram was then cut and eluted with 50% ethanol. The ethanol solution was dried in a planchet and counted. It gave 712 cpm.

The remaining solution containing $1.88 \text{ mg} (2.36 \times 4/5)$ of isovalthine was dried and the residue was dissolved again in 0.1 ml of water. Each 0.02 ml of the solution was developed two dimensionally on five filter papers by the method of UBUKA³. From three chromatograms, isovalthine areas were cut and extracted together with water. The water extract was dried in a planchet and counted. The dried matter weighing 0.7 mg gave 1.271 cpm. The dried matter in a planchet was dissolved in a small amount of water and was determined for isovalthine content on amino acid analyzer. The amount of isovalthine in the 0.7 mg dried matter was 0.46 μ moIe (0.1 mg) instead of 1.12 mg which was the calculated







Experimental Isovalthinuria

amount of isovalthine remaining at this stage. Thus the treatments such as chromatography, extraction, or drying have caused the decomposition or loss of isovalthine, and alanine and some unknown peaks appeared on the analyzer chart.

Although the specific activity of S^{35} -isovalthine could not be accurately calculated, it might be assumed to be over 10,000 cpm per mg in this case.

The remaining two paper chromatograms were used for autoradiography. Fuji X-ray film Type 200 was used for this purpose. Fig. 1 shows the chromatogram and its autoradiogram. Thus the sulfur atom of cystine has definitely been incorporated into urinary isovalthine.

3. S³⁵-Methionine Group

Total volume of urine on the 6th week was 620 ml. The amount of isovalthine in the acidic amino acid fraction was 10.8 μ moles (2.38 mg).

The isovlathine fraction collected by a fraction collector was dried in a planchet after deionized as described above. The dried matter weighing 26.38 mg, which should contain 2.14 mg (2.38 mg \times 9/10) of isovalthine, showed 9,358 cpm.

The dried matter in the planchet was dissolved in 0.1 ml of water and 0.02 ml of the solution was developed one dimensionally on a paper strip by electrophoresis and colored with ninhydrin. The chart of paper chromatogram scanner showed a somewhat distinct peak of isovalthine but the starting line again showed the highest peak. The isovalthine band was cut from the chromatogram and eluted with 50 % ethanol and dried in a planchet. It showed 308 cpm.

The remaining solution was developed two dimensionally on five papers as in the case of S³⁵-cystine. The isovalthine containing dried matter prepared from three chromatograms was 0.9 mg and showed 372 cpm. The dried matter was found to contain 1.64 μ moles (0.36 mg) of isovalthine on amino acid analyzer instead of 1.02 mg which was the calculated amount remaining at this stage. Much isovalthine was also lost.

Specific activity of isovalthine in this case may be assumed to be around 1,000 cpm per mg. So the sulfur atom of methionine is utilized less for isovalthine synthesis than that of cystine.

Remaining two paper chromatograms are used for the preparation of autoradiogram which is shown in Fig. 2.

DISCUSSION

The data obtained here are not so beautiful, but it certainly proves the origin of sulfur atom of urinary isovalthine.

According to the in vitro experiments of KUWAKI et al., isovalthine is at

5

70 T. UBUKA, K. HORIUCHI, T. SHIMOMURA, and S. MIZUHARA

first synthesized in liver as glutathione-isovaleric acid conjugate (GSIV)³ and GSIV is converted into isovalthine by kidney homogenate^{*}. Although the specific activity of urinary isovalthine could not be accurately calculated, the results appear to indicate that cysteine is more direct source for the synthesis of gluta-thione and again of isovalthine than methionine.

SUMMARY

In the course of experimental isovalthinuria induced by cholic acid, S³⁵methionine or S³⁵-cystine administered was incorporated into urinary isovalthine in guinea pigs.

Sulfur atom of cysteine seems to be utilized much better for isovalthine synthesis than that of methionine.

ACKNOWLEDGEMENT

We are indebted to Prof. M. YAMAMOTO of Department of Radiation Medicine for his help in preparing autoradiography. This work was supported by research grants from U. S. National Institutes of Health (HE-07419), and the Ministry of Education of Japan.

REFERENCES

- 1. Ubuka, T.: Acta Med. Okayama 17, No. 6, 1963 in press
- 2. UBUKA, T.: J. Biochem. 52, 440, 1962
- 3. KUWAKI. T., OHMORI, S., and MIZUHARA, S.: Biochim. Biophys. Acta 78, 553, 1963

^{*} Unpublished data of this laboratory.