CHANGES IN POLYAMINE METABOLISM IN RAT LIVER AFTER ORAL ADMINISTRATION OF ALPHA-NAPHTHYLISOTHIOCYANATE

Yoshikazu AOKI, Hideo SUZUKI and Hajime ITOH*

Department of Clinical Chemistry, Kitasato University School of Hygienic Sciences,
15-1 Kitasato 1 Chome, Sagamihara, Kanagawa 228, Japan

Accepted January 6, 1986

Abstracts.....Changes in the levels of urea cycle enzymes and polyamine metabolism in the liver of rats treated with α -naphthylisothiocyanate (ANIT), an inducer of experimental cholestasis, were studied. Activities of arginase increased approximately two-fold compared to the control values during the period of 24-72 hours after oral administration of ANIT (100 mg/kg), while activities of ornithine carbamyltransferase and ornithine aminotransferase decreased. The activity of ornithine decarboxylase was elevated by approximately 20- and 10-fold at 12 and 60 hours, respectively, after ANIT administration. Putrescine concentration doubled 24-48 hours after the ANIT administration, but spermidine level rose more slowly and reached the level of 1.5-fold of the control level in 36-72 hours. Spermine concentration decreased initially but increased in 96 hours. These results suggest that the increased activity of urea cycle accounts for the increase in the ornithine content and that the putrescine and spermidine acts as the initiator of recovery of the liver damaged by ANIT treatment.

Key word : Polyamines, putrescine, spermidine, arginase, ornithine decarboxylase, ANIT

INTRODUCTION

The intracellular contents of polyamines are high in secretive organs such as pancreas, prostate gland, and thymus and in bone marrow where synthetic activities of nucleic acids and proteins are high (Jänne, 1964). In the actively growing tissues, the polyamine levels are higher than in non-growing tissues, (Raina et al., 1975 ; Synder et al., 1970). The synthesis of polyamines is known to increase after the tissue is subjected to surgical treatment such as hepatectomy, wounding, and administration of drugs, or also known to increase in the cases of appearance of various neoplastic cells. The polyamine synthesis increases at the incipient stage of the cell proliferation or cellular

連絡先：〒228 相模原市北里1丁目15-1 北里大学衛生学部臨床化学教室 伊藤 啓

*To whom correspondence should be addressed.

restoration before the elevation of nucleic acid synthesis. Thus, it has been suggested that physiological function of polyamines seems to be the initiator of the proliferation of cells (Raina et al., 1975; Synder et al., 1970; Augustine et al., 1980).

Administration of α -naphthylisothiocyanate (ANIT) in rats induces severe cholestatic liver damage, as the result of denaturation of the epithelium of bile caliculi and the obstruction of the biliary tract by necrosis (Fukumoto, 1978; Leonard et al., 1981, 1984). Recently, attention has been drawn to the disorder of liver cell such as the abnormality of the bile acid metabolism, and ultrastructural deviation of endoplasmic reticula or mitochondria by administration of ANIT in rats (Schaffner et al., 1973; Czygan et al., 1974).

In this paper, we studied the amount of polyamine levels during the course of recovery of the liver, and the changes in the enzymes of urea cycle which participate in the polyamine synthesis in rats with causing the cholestatic disorder after ANIT administration.

MATERIALS AND METHODS

Reagents and Equipment: ANIT was a product of Kanto Chemical Co. (Tokyo). Carbamyl phosphate was purchased from Boehringer Mannheim Yamanouchi (Tokyo). [$1\text{-}^{14}\text{C}$]-Ornithine hydrochloride (56 mCi/mmol) was from Radiochemical Centre (Amersham). Dansyl chloride, spermine and *o*-aminobenzaldehyde were from Nakarai Chemicals (Kyoto). Spermidine and putrescine were from Sigma Chemical Co. (St. Louis). The other reagents were analytical grade and obtained from Wako Pure Chemical Industries (Osaka).

The high performance liquid chromatography (HPLC) was performed using Hitachi 633 A, with Hitachi 635-0420 gradienter. An HPLC column was Micro Pack CN-10 (4×300 mm) purchased from Varian Instrument Division (Calif.). Clinalyzer SI-6 from JEOL (Tokyo) was used for the analysis of serum components.

Animals: Male rats of the Wister strain, 250-300 g body weight (b.w) were used throughout. The animals were given laboratory chow and water *ad libitum*. Food was removed 24 hour before administration of ANIT. ANIT was dissolved in olive oil at the concentration of 100 mg/ml, and it was given orally at 100 mg/kg b.w. Control animals were given 1 ml/kg b. w. of olive oil.

A group of five rats was used in each experiment. The rats were sacrificed under barbital anesthesia at 12, 24, 36, 48, 60, 72, and 96 hours after oral administration of ANIT, and blood was collected from abdominal vein for assays of several serum components. The livers were removed and perfused with ice-cold saline, weighed and then divided into parts for assay of enzyme activities and polyamine contents.

Enzyme assays: Livers were homogenized with a Polytron homogenizer in 10 ml/g of liver of ice-cold M/15 phosphate buffer (pH 7.4). The homogenate was centrifuged at $24,000 \times g$ for 60 minutes at 4°C , and the supernatant was used for enzyme assays.

Arginase [EC 3.5.3.1] activity was determined by the method of Schimke (1970),

ornithine carbamyltransferase [EC 2.1.3.3] was by the method of Nakamura et al. (1970), and ornithine aminotransferase [EC 2.6.1.13] was by Jenkins et al. (1970). The activity of ornithine decarboxylase [EC 4.1.1.17] was determined by measuring the rate of liberation of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]\text{-L-ornithine}$. Aspartate aminotransferase [EC 2.6.1.1; GOT] and alanine aminotransferase [EC 2.6.1.2; GPT] activities in serum were determined by the method of Karmen (1955). Alkaline phosphatase [EC 3.1.3.1; ALP] activity in serum was determined by the Kind-King method (Kind et al., 1954).

Determination of polyamines and other components: Polyamines were determined by high performance liquid chromatography according to the method of Newton et al. (1976). Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Total cholesterol concentration in serum was determined by using *o*-phthalaldehyde (Naka, 1972). Bilirubin concentration was determined by the method of Malloy Evelyn (Malloy et al., 1937). The excreted amount of bile was measured for 25 minutes by cannulating into the common bile duct of rats.

RESULTS

Changes in the contents of some components and the excretory amount of the bile after ANIT administration :

The activity of GOT began to increase within 12 hours after the administration, and reached the level five times higher than control level after 36 to 48 hours, and returned to the control level after 96 hours. The changes in GPT activity were similar to those in GOT. It increased to fourteen times the control level after 24 to 48 hours. After administration of ANIT, the concentration of cholesterol and bilirubin and ALP activity rose to five to twelve times (Fig. 1). They were excreted into the bile, but transferred to serum as the results of the impediment of excretion.

When the excretory amount of the bile was measured after ANIT administration in rats, the control level was recovered after 72 hours (Fig. 2). In these experiments, we also established that the cholestatic liver injury had occurred in 24 to 48 hours after ANIT administration. Judged from the fact the activities of GOT and GPT were increased at the same time as elevation of ALP activity and bilirubin concentration, the hepatocyte as well as cholangiole was injured at the same time.

Enzyme activities in the urea cycle in rat liver: Changes in the arginase activity after ANIT administration was shown in Fig. 3. The activity of arginase rose by 1.5 times in 24 hours ($p < 0.1$). These findings suggested the accelerated synthesis of ornithine from arginine.

On the other hand, ornithine carbamyltransferase activity decreased between 12 and 24 hours after administration of ANIT (Fig. 4a), and the low activity was maintained from 24 to 72 hours ($p < 0.01$), and recovered in 96 hours to the control level. The activity of ornithine aminotransferase, which catalyzes conversion of ornithine to glutamate γ -semialdehyde, decreased gradually, and kept low until 96 hours (Fig. 4b).

These findings suggest that the ornithine accumulates in the liver as the tissue

Fig. 1 Time-course changes in the several components in serum of rats after ANIT administration. ANIT, 100 mg/kg b. w., was given orally. The enzyme activities and concentration of cholesterol and bilirubin were determined according to the procedure described in MATERIALS AND METHODS. Each plot indicates mean $\pm$ SD.

Changes in polyamines after ANIT administration

Fig. 2 Time-course changes in bile excretion after ANIT administration . ANIT at a dose of 100 mg/kg b. w. was given orally. Each plot indicates mean $\pm$ SD. The procedure is described in MATERIALS AND METHODS.

Fig. 3 Time-course changes in the specific activities of arginase in the liver of rats after ANIT administration. ANIT, 100 mg/kg b. w., was given orally. Each plot indicates meas $\pm$ SD. *p<0.1.

Fig. 4a-4b Time-course changes in the specific activities of ornithine carbamyltransferase and ornithine aminotransferase in the liver of rats after ANIT administration.

ANIT, 100 mg/kg b. w., was given orally. Fig. 4a shows the changes of ornithine carbamyltransferase specific activity (—●—), and Fig. 4b shows that of ornithine aminotransferase (—■—). Each plot indicates mean $\pm$ SD. * $p < 0.01$.

recovers from the injury.

Changes in ornithine decarboxylase activity and polyamine levels in the liver after ANIT administration in rats :

The specific activity of ornithine decarboxylase which catalyzes the formation of putrescine from ornithine increased in 12-72 hours after ANIT administration (Fig. 5). It increased by 18 times in 12 hours and by 12 times in 72 hours ($p < 0.01$).

After administration of ANIT putrescine in the liver rose in 24 hours to the highest level that was twice as high as that of the control in 48 hours ($p < 0.01$), then decreased to the control level in 96 hours (Fig. 6a). When the spermidine concentration in the liver was compared with the control value, it increased by more than 1.5 times between 36 and 72 hours after ANIT administration (Fig. 6b). However spermine concentration showed no significant changes during the first 12 hours and then decreased to about 30

Changes in polyamines after ANIT administration

Fig. 5 Time-course changes in ornithine decarboxylase specific activity in the liver in rats after ANIT administration.

ANIT, 100 mg/kg b. w., was given orally. Each plot indicates mean $\pm$ SD.

* $p < 0.01$.

Fig. 6a-6c Time-course changes in polyamine levels in the liver after ANIT administration. ANIT, 100 mg/kg b. w., was given orally. The changes of putrescine concentration is showed in Fig. 6a(—●—), spermidine concentration in Fig. 6b (—■—), and spermine concentration in Fig. 6c (—▲—), respectively. Each plot indicates mean $\pm$ SD. * $p < 0.01$, ★ $p < 0.05$.

-40% of the control level in 24 to 60 hours ($p < 0.05$). The time course of the spermine concentration was a mirror image of that of the spermidine concentration (Fig. 6c).

DISCUSSION

The mechanism of the cholestatic liver injury by administration of ANIT has been examined in detail by Fukumoto (1978) and Leonard et al. (1981). ANIT is converted to an activated substance by the microsomal drug metabolizing system containing cytochrome p-450 in the liver, and it obstructs the excretion of the bile acids in the bile canaliculi. Thus it causes a functional and morphological disorder as a result of necrosis and then impairment of epithelia of biliary canaliculi, which forms the obstruction (Fukumoto, 1978). Besides, Leonard et al. (1981) demonstrated that ANIT increases hepatic epoxide hydrolase activity. While the effects of ANIT are not attributable to the direct effects of bile salts, in particular the detergent properties of bile on epoxide hydrolase, increase epoxide hydrolase activity correlated well with the extent of ANIT-dependent bile duct proliferation. Furthermore, bile duct ligation causes bile duct proliferation and increases in epoxide hydrolase activity which show a remarkable similarity to the effects of ANIT treatment. When cholestasis is induced by ANIT administration in rats, the activity of arginase which catalyze the biosynthesis of ornithine from arginine was increased (Fig. 3), suggesting the increased synthesis of ornithine. In contrast, the activities of ornithine carbamyltransferase and ornithine aminotransferase which metabolize the ornithine to citrulline or glutamate γ -semialdehyde decreased after ANIT administration. Thus these results account for the accumulation of ornithine in the liver.

Ornithine decarboxylase which is the key enzyme in the synthesis of polyamines increased biphasically. This is because the first phase is induced by the endogenous growth hormone, and because the second phase is induced by during the liver recovery (Höltta and Jänne, 1972). Kasai et al. (1982) demonstrated that the urea synthesis was superior to the polyamine synthesis in the mature liver, but in the fetal liver, polyamines and pyrimidine synthesis were more active to urea, as in the case of a regenerating liver. The activity of ornithine decarboxylase increases at the incipient stage in fetus, and the urea synthesis increases with growth, but the activity of ornithine decarboxylase decreases.

Putrescine increased at the beginning after the liver injury which is followed by increase in spermidine. Spermidine did not decrease until 96 hours ($p < 0.01$). On the other hand, spermine decreased at the beginning ($p < 0.05$), and increased later. Russell and Lombardini (1971) have reported in the test of regenerating liver that putrescine increased after the operation, and that it doubled in 24 hours. In contrast, spermine decreased after the hepatectomy, and it recovered to the control value in 14 days. This result was similar to our study. They have also reported that after the hepatectomy the amounts of putrescine and spermidine reach the maximum value within 48 hours before

the RNA concentration approaches maximum.

When the cholestatic liver injury was induced by ANIT administration, the activities of the enzymes of the urea cycle in the liver were altered such that the ornithine synthesis was increased. In addition ornithine decarboxylase was activated resulting in accelerated synthesis of putrescine and spermidine (Fig.7). These results showed firmly that the recovery of the tissue after the injury was initiated by putrescine and spermidine.

Fig. 7 Scheme of the changes of metabolism in urea cycle and polyamines after ANIT administration ←←← indicates the increase of enzyme activity, and →→→ indicates the decrease of activity. (↑) indicates the increase of polyamine contents, and (↓) indicates the decrease of the contents.

ACKNOWLEDGEMENT

We thank Prof. K. Uyeda of the University of Texas for helpful comments on the manuscript.

REFERENCES

- Augustine, S. L. and Swick, R. W. (1980) : Turnover of total proteins and ornithine aminotransferase during liver regeneration in rats. *Am. J. Physiol.*, **238** (Endocrinol, Metab. 1), E 46-E 52.
- Czygan, P., Greim, H., Hutterer, et al. (1974) : Comparison of two types of intrahepatic jaundice in rats with bile duct ligation. *Acta Hepato-Gastroenterol.*, **681**, 339-345.

- Fukumoto, Y. (1978) : Studies on alpha-naphthylisothiocyanate induced cholestasis. *Liver*, **19**, 647-656.
- Hölttä, E. and Jänne, J. (1972) : Ornithine decarboxylase activity and the accumulation of putrescine at early stages of liver regeneration. *FEBS Lett.*, **23**, 117-121.
- Jänne, J., Raina, A. and Sjimes, M. (1964) : Spermidine and spermine in rat tissues at different ages. *Acta Physiol. Scand.*, **62**, 352-358.
- Jenkins, W. T. and Tsai, H. (1970) : Ornithine aminotransferase (Pig kidney). *Methods in Enzymology*, vol. VXL-A, p. 281-285, Academic Press, New York.
- Karmen, A. (1955) : A note on the spectrophotometric assay of glutamic-oxalacetic transaminase in human blood serum. *J. Clin. Invest.*, **34**, 131-133.
- Karsai, T. and Elödi, P. (1982) : Urea cycle enzymes in human liver ; ontogenesis and interaction with the synthesis of pyrimidines and polyamines. *Mol. Cell. Biochem.*, **43**, 105-110.
- Kind, P. R. and King, E. J. (1954) : Estimation of plasma phosphatase by determination of hydrolyzed phenol with aminoantipyrine. *J. Clin. Pathol.*, **7**, 322-326.
- Leonard, T. B., Popp, J. A., Graichem, M. E., et al. (1981) : α -Naphthylisothiocyanate induced alterations in hepatic drug metabolizing enzymes and liver morphology : Implications concerning anticarcinogenesis. *Carcinogenesis*, **2**, 473-482.
- Leonard, T. B., Neptun, D. A., and Popp, J. A. (1984) : Serum gamma glutamyl transferase as a specific indicator of bile duct lesions in the rat liver. *Am. J. Pathol.* ; **116**, 262-269.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., et al. (1951) : Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265-275.
- Malloy, H. T. and Evelyn, K. A. (1937) : The determination of bilirubin with the photoelectric colorimeter. *J. Biol. Chem.*, **119**, 481-490.
- Naka, H. (1972) : Serum cholesterol determination with o-phthalaldehyde reagent. *Jap. J. Clin. Pathol.*, **20**, 291-294.
- Nakamura, M. and Jones, M. E. (1970) : Ornithine carbamyltransferase (*Streptococcus faecalis*). *Methods in Enzymology*, vol. XVII-A, p. 286-295, Academic Press, New York.
- Newton, N. E., Ohno, K. and Abdel-Monem, M. M. (1976) : Determination of diamines and polyamines in tissues by high-pressure liquid chromatography. *J. Chromatogr.*, **124**, 277-285.
- Raina, A. and Jänne, J. (1975) : Physiology of the natural polyamines putrescine, spermidine and spermine. *Med. Biol.*, **53**, 121-147.
- Russell, D. H. and Lombardini, J. B. (1971) : Polyamines : (1) Enhanced s-adenosyl-l-methionine decarboxylase in rapid growth systems, and (2) The relationships between polyamine concentrations and RNA accumulation. *Biochim. Biophys. Acta*, **240**, 273-286.
- Schaffner, F., Scharnbeck, H. H., Hutterer, F., et al. (1973) : Mechanism of cholestasis VII. α -naphthylisothiocyanate-induced jaundice. *Lab. Invest.*, **28**, 321-331.
- Shimke, R. T. (1970) : Arginase (Rat liver). *Methods in Enzymology*, vol. XVII-A, p. 313-317. Academic Press, New York.
- Snyder, S. H. and Russell, D. H. (1970) : Polyamine synthesis in rapidly growing tissues. *Fed. Proc.*, **29**, 1575-1582.