THE BEHAVIOR OF PENTAERYTHRITOL TETRANICOTINATE IN RAT GASTROINTESTINAL TRACT AS A PRODRUG

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The gas chromatographic assay method for pentaerythritol tetranicotinate, a nicotinic acid prodrug, and its hydrolysates was developed. The behavior of the drug in gastrointestinal tract was investigated in rat by using the method. The disappearance and hydrolysis of the drug were not observed in the gastric loop until 30 min. The rate of disappearance from the intestinal loop was 36.7% at 30 min which was significantly smaller than that of nicotinic acid. Little hydrolysis of the drug was observed in the buffer solution, pH 7.4, at 37° up to 2 hr. However, the consecutive hydrolysis was observed when the drug was incubated with everted intestine or plasma. As to the rate of hydrolysis of the drug and its ester-form hydrolysates by scraped intestinal mucosa, the ester to which the larger number of nicotinic acid was bound was hydrolyzed more rapidly. These results indicate that the orally administered drug is enzymatically hydrolyzed in the intestinal mucosa by a consecutive reaction. Although the hydrolysis rate of pentaerythritol tetranicotinate is rapid, the rate of its ester-form hydrolysate becomes slower gradually as the nicotinic acid is released. The released nicotinic acid is rapidly absorbed. The behavior of the drug revealed in this study suggests that pentaerythritol tetranicotinate is useful as a prodrug of nicotinic acid.

Keywords—pentaerythritol tetranicotinate; nicotinic acid; prodrug; absorption; enzymatic hydrolysis; gas chromatographic assay; gastrointestinal

INTRODUCTION

It has been reported that nicotinic acid reduced the concentration of blood lipids such as cholesterol, triglycerides, and phospholipids in hyperlipemia.¹⁾ However, a large dose of nicotinic acid (3–6 g/day) is necessary to maintain an effective plasma concentration because of its rapid elimination from blood.^{1b)} Subsequently the rapid increase of plasma concentration frequently induces the side effects such as flushing and gastrointestinal disturbances.²⁾ Therefore, the clinical use of nicotinic acid has been limited.

In order to solve these shortcomings, *i.e.* to prolong the pharmacologically effective plasma concentration and prevent the side effects, several derivatives of nicotinic acid have been examined.³⁾ Pentaerythritol tetranicotinate (PETN), the structure is shown in Fig. 1, is a prodrug which releases nicotinic acid as an active

metabolite. A moderate but significant increase of the plasma nicotinic acid concentration and its duration after oral administration of PETN were reported,⁴⁾ and the fate of PETN has been investigated in men,⁵⁾ rats,⁶⁾ rabbits,⁷⁾ and cats.⁸⁾ The ester-form metabolites, pentaerythritol trinicotinate (Tri), dinicotinate (Di), and mononicotinate (Mono), were detected in rat serum after oral administration of PETN.⁶⁾ However, it has not been shown clearly at where these ester-form metabolites are produced, that is, at where PETN is hydrolyzed to release nicotinic acid.

In order to clarify the factors contributing toward the appropriate profile of plasma nicotinic acid concentration after oral administration of PETN as stated above, it is worthwhile to investigate its mode of hydrolysis in the gastrointestinal tract. It is considered to be one of the useful approach to achieve a better pharmaceutical

FIG. 1. Structure of Pentaerythritol Tetranicotinate (PETN)

design to study the behavior of PETN in the gastrointestinal tract in this manner.

For studying the behavior of PETN in the gastrointestinal tract, a new analytical method was developed. By using the newly developed gas chromatographic method, PETN and its hydrolyses (Tri, Di, Mono, pentaerythritol, and nicotinic acid) were assayed, and the behavior of PETN in the gastrointestinal tract was investigated.

MATERIALS AND METHODS

Materials — Pentaerythritol tetranicotinate (Niceritrol, Perycit[®]), trinitocinate, dinicotinate, and mononicotinate were supplied from Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya. Nicotinic acid and pentaerythritol were purchased from Kishida Chemical Co., Ltd., Osaka and Wako Pure Chemical Industries Co., Ltd., Osaka, respectively, and trimethylchlorosilane and hexamethyldisilazane were obtained from Tokyo Kasei Kogyo Co., Ltd., Tokyo. All the chemicals were of reagent grade and used without further purification.

Animals — Wistar male rats weighing about 250 g were used as the experimental animals. They were anesthetized with sodium pentobarbital after fasting 15–18 hr.

Disappearance of PETN from Gastric and Intestinal Loops — 1) Gastric Loop: Pentaerythritol tetranicotinate was dissolved in simul-

ated gastric juice (4.55×10^{-4} M, JP IX degradation medium No. 1). After washing the inside of the stomach with saline solution, the cardia and pylorus were ligated and 1 ml of the drug solution was injected into the loop. After 30 min, the loop was removed. The contents of the loop and 2 ml of simulated gastric juice with which the inside of the loop was rinsed were mixed.

2) Intestinal Loop: Pentaerythritol tetranicotinate was suspended in modified Krebs bicarbonate buffer solution, pH 7.4^{9)*} (4.55×10^{-4} M). Three 10 cm loops at intervals of 1 cm were prepared beginning 10 cm from pylorus.¹⁰⁾ One milliliter of the drug suspension was injected into the loop. The successive operation was the same as that described in the gastric loop except rinsing with a modified Krebs bicarbonate buffer solution.

Hydrolysis of PETN — 1) Hydrolysis in Buffer Solutions: An Erlenmeyer flask containing 50 ml of 4.55×10^{-4} M PETN in simulated gastric juice was immersed in a water bath maintained at 37° and shaken. The solution was continuously bubbled with 5% carbon dioxide in oxygen. At suitable intervals, 1-ml specimen was pipetted and assayed for the drug. When the hydrolysis of PETN in modified Krebs bicarbonate buffer solution (pH 7.4) was studied, the initial concentration of the drug was 2.5×10^{-5} M because of the low solubility at this pH, therefore, a 5 ml-specimen was pipetted.

2) Hydrolysis by Everted Intestines: After removal of the whole small intestine, the first 15 cm segment was discarded. The next 45 cm segment was rinsed with cold saline solution, everted, and divided into three 15 cm segments. The serosal side was filled with 1.5 ml of modified Krebs bicarbonate buffer solution. The sac was placed in an Erlenmeyer flask containing 40 ml of 2.5×10^{-5} M PETN in modified Krebs bicarbonate buffer solution. It was incubated at 37° in the same way as the studies using buffers. Five milliliters of the mucosal solution was pipetted

* 80 parts of 0.9% NaCl, 21 parts of 1.3% NaHCO₃, 4 parts of 1.15% KCl, 1 part of 2.11% KH₂PO₄, 1 part of 3.82% MgSO₄ · 7 H₂O, 1 part of 1.62% CaCl₂ · 2 H₂O.

at 15, 30, and 60 min. One milliliter of the serosal solution at 60 min was examined for the permeability through the intestine.

3) Hydrolysis of PETN and Its Ester-form Hydrolysates by Scraped Mucosa of Small Intestine: The first 40 cm segment of the small intestine from the pylorus was removed. After rinsing with cold saline solution, the mucosa was scraped on ice blocks with a slide glass and suspended in cold modified Krebs bicarbonate buffer solution to make 10 ml (A).¹¹⁾ One milliliter of (A) was diluted to 20 ml with the same buffer solution (B). To 100 ml of each prewarmed ester solution (2.5×10^{-5} M in modified Krebs bicarbonate buffer solution containing 1% ethanol), 4 ml of (A) or (B) was added. (A) was for Mono and Di, and (B) for Tri and PETN. They were incubated at 37° in the same way as stated above. At suitable intervals, a 5-ml specimen was pipetted through a cotton plug.

4) Hydrolysis in Rat Plasma: One milliliter of plasma was placed in each glass-stoppered test tube and prewarmed at 37°. Fifty microliters of PETN dimethyl sulfoxide solution was added to each tube (a final concentration of PETN was 2×10^{-4} M), and incubated for predetermined period.

Procedures for Assay of PETN and Its Hydrolysates — Pentaerythritol tetranicotinate and its hydrolysates were measured primarily by gas chromatography (GLC) and partly by high performance liquid chromatography (HPLC).

1) Disappearance of PETN from the Loop: One milliliter of distilled water and 0.5 ml of hydrochloric acid were added to the mixture of the content of the loop and rinsed-out solution. After the complete hydrolysis of esters to nicotinic acid and pentaerythritol by reflux in an oil bath for 5 hr, distilled water was added to make 5 ml. One milliliter of the solution was dried *in vacuo* on a water bath, the residue was assayed for the drugs remaining in the loop for total nicotinic acid with GLC. The unchanged PETN remained in the gastric loop was assayed as follows: To the mixture of the content and rinsed-out solution 0.2 M acetate buffer solution (pH 5.0)

was added to make 5 ml. One milliliter of distilled water was added to 4 ml of the solution and extracted with 5 ml of acetone-chloroform (2:3 v/v). The extraction ratio was constant in the pH range of 4–8. One milliliter of the organic layer was evaporated to dryness *in vacuo* and the residue was assayed with GLC.

2) Hydrolysis of PETN: The typical method for measurement of PETN and its hydrolysates in the incubation media is shown in Chart 1. After addition of 7 ml of acetone-chloroform mixture to 5 ml of the sample, the mixture was shaken and centrifuged. Five milliliters of the organic layer was taken to dryness *in vacuo* and the residue was assayed for PETN, Tri, and Di with GLC. On the other hand, 0.2 ml of 0.1 M barium chloride was added to 1 ml of aqueous layer to precipitate phosphoric acid in buffer solution, as barium salt, which will disturb the gas chromatographic assay. After centrifugation, 0.1 ml of 0.4 N hydrochloric acid was added to 1 ml of the supernatant to make nicotinic acid the non-ionic form. The mixture was freeze-dried (Meiwa MW-400 freeze drier) and the residue was assayed for Mono, pentaerythritol, and nicotinic acid.

When the hydrolysis of PETN in simulated gastric juice was examined, 4 ml of 0.2 M acetate buffer was added to 1 ml of sample and extracted with 5 ml of acetone-chloroform. One milliliter of the organic layer was taken to dryness.

The drugs in plasma were measured as follows: One milliliter of distilled water and 3 ml of acetone-chloroform were added to the incubation mixture. After shaking and centrifugation, 2 ml of the organic layer was taken to dryness. And 1 ml of the aqueous layer was treated in the same way as above.

Freeze-dried residue of 0.5 ml of aqueous layer after extraction with acetone-chloroform was subjected to the assay for Mono and nicotinic acid by HPLC.

3) Gas Chromatographic Assay: The hydrolysates of PETN were assayed as trimethylsilyl derivatives. The residue of the organic layer was dissolved in 20 μ l of the internal standard (dioctyl phthalate) pyridine solution. To 10 μ l of the solu-

tion, 5 μ l of trimethylsilylating (TMS) agent (hexamethyldisilazane : trimethylchlorosilane = 5 : 2) was added and the mixture was heated at 60° for 30 min. Two microliters of the reaction mixture was injected into GLC. The residue of the aqueous layer was dissolved in 10 μ l of the

CHART 1. *Procedures for Assay of PETN and Its Hydrolysates*

FIG. 2. *Gas Chromatograms of PETN and Its Hydrolysates*

1, pentaerythritol; 2, nicotinic acid; 3, internal standard; 4, Mono; 5, internal standard; 6, Di; 7, Tri; 8, PETN.

internal standard (diphenylamine) pyridine solution and 70 μ l of TMS agent and heated at 60° for 30 min. Four microliters of the reaction mixture was injected into GLC.

Gas Chromatographic Conditions: For PETN, Tri, and Di—The gas chromatograph (HITACHI 063) equipped with a flame ionization detector was run using a glass column (2 m $\times$ 3 mm i.d.) packed with 1% Silicone OV-1 on 60–80 mesh Chromosorb W AW DMCS. The oven, injection port, and detector temperatures were 200–290° (20°/min), 320°, and 320°, respectively. The flow rate of nitrogen was 20 ml/min. The representative gas chromatogram is shown in Fig. 2. For mono, pentaerythritol, and nicotinic acid—A glass column (2 m $\times$ 3 mm i.d.) packed with 1.5% Silicone XE-60 on 80–100 mesh Chromosorb G.H.P was used. The oven, injection port, and detector temperatures were 50–190° (20°/min), 230°, and 230°, respectively. The flow rate of nitrogen was 45 ml/min. The representative gas chromatogram is shown in Fig. 2. The peak area was calculated by using a digital integrator (Takeda Riken, TR-2221A).

4) High Performance Liquid Chromatographic Assay: The residue was dissolved in 100 μ l of the internal standard (metharbital) aqueous solution. After centrifugation, 20 μ l of the supernatant was injected into HPLC.

Chromatographic Conditions;¹²⁾ A liquid chromatograph (HITACHI 635A) equipped with a high pressure sampling valve (635-0650, 1 μ l–2.0 ml) and a multi-wavelength UV detector (HITACHI 635M) was used. The column, 25 cm $\times$ 4 mm i.d. stainless steel, was packed with Licosorb RP-18, 5 μ m (E. Merck) and warmed at

55° using a water bath circulator. The mobile phase was 2.0×10^{-3} M dioctyl sodium sulfosuccinate aqueous solution adjusted the pH to 2.5 with 10% formic acid and methanol, 2 : 1. It was deaerated prior to use. The flow rate was 1.0 ml/min. The wavelength and absorbance unit full scale were 260 nm and 0.01, respectively.

The peak height ratio was calculated for determination of drug concentrations.

RESULTS AND DISCUSSION

Stability of PETN in Buffer Solution

Little hydrolysis of PETN was observed in modified Krebs bicarbonate buffer, pH 7.4 and simulated gastric juice at 37° during 2 hr incubation period, although a very small peak of Tri was detected on a gas chromatogram 30 min after at pH 7.4. These findings are in accordance with those reported previously.^{5,13)}

Behaviors of PETN in Gastrointestinal Tract

The disappearance of PETN from the gastric loop was not observed until 30 min. The drug recovered from the loop was $101.7 \pm 7.5\%$ (mean $\pm$ S.D., $n=3$) as nicotinic acid. Furthermore, upon a direct analysis of the recovered solution without hydrolysis, the concentration of PETN did not decrease and no other hydrolysates were detected. It is considered that these results indicate no absorption and hydrolysis of PETN in the stomach.

Table I shows the disappearance of PETN from the intestinal loop at 30 min in comparison with that of nicotinic acid. Pentaerythritol tetranicotinate was administered as a suspension, while the equimolar nicotinic acid was used as a solution. Nicotinic acid rapidly disappeared from the

TABLE I. *Disappearance of PETN and Nicotinic Acid from Rat Intestinal Loops*

	Percent of disappearance as nicotinic acid	Significance level
Nicotinic acid	59.3 ± 10.5	
PETN	36.7 ± 14.3	$p < 0.01$

*Drug concn.: 1.82 mM as nicotinic acid.
Mean $\pm$ S.D. ($n=6$).*

loop, and the percent of disappearance of PETN was significantly smaller than that of nicotinic acid. The rapid absorption of nicotinic acid is well-known.^{1b,4,14)} Therefore, once PETN is hydrolyzed to release nicotinic acid in the intestine, a rapid absorption of the released nicotinic acid can be predicted.

The hydrolysis profile of PETN by the everted small intestine is shown in Fig. 3. The percent of drug remained is represented as the nicotinic acid bound to each ester. It was observed that each hydrolysate was consecutively produced with the rapid decrease of PETN. The observed hydrolysis is considered to be enzymatic^{5,15)} because it was not observed in the same buffer solution in the absence of everted intestine.

The percent of nicotinic acid recovered in the serosal medium was $2.4 \pm 1.3\%$ (mean $\pm$ S.D., $n=6$) after 60 min. However, PETN and other hydrolysates were not detected in the medium. Estimating from the limits of detection, the percent of the esters recovered in the serosal medium were, if any, less than 0.3, 0.2, 0.1, and 0.04% as

FIG. 3. Hydrolysis Profile of PETN by Rat Everted Intestine

○, PETN; ●, Tri; △, Di; ●, Mono; ▲, nicotinic acid. Each point represents the mean of 6 experiments with S.D. The lines are drawn to fit the points as closely as possible.

the bound nicotinic acid for PETN, Tri, Di, and Mono, respectively.

The hydrolysis of PETN, Tri, Di, and Mono by scraped mucosa of small intestine was investigated. A linear relationship was obtained when the logarithm of the percent of each ester remained was plotted against the incubation time as shown in Fig. 4. The incubation media contained 1% ethanol which was used as the solvent of the stock solutions of the esters. In a preliminary experiment, it was observed that the presence of ethanol tended to slow the hydrolysis rate of PETN only slightly, and it did not affect the half-life of PETN in hydrolysis by scraped mucosa. Under the present experimental conditions, the half-lives of PETN, Tri, and Di calculated from the slopes were 5.7, 19.7, and 28.8 min, respectively. The half-life of Di was longer than those of PETN and Tri and little hydrolysis of Mono was observed in spite of 20 times amount of scraped mucosa added. The ester to which the larger number of nicotinic acid were bound was hydrolyzed more rapidly.

Stability of PETN in Plasma

Figure 5 shows the hydrolysis profile of PETN in rat plasma. Dimethyl sulfoxide was added to the plasma in a final concentration of about 5% to facilitate the dissolution of PETN because of its

FIG. 4. Hydrolysis of PETN and Its Ester-Form Hydrolysates by Scraped Mucosa of Rat Intestine
○, PETN; ●, Tri; △, Di. Each point represents the mean of 4 experiments.

low solubility at the pH of the plasma.⁵⁾ It was reported that the hydrolysis rate of PETN was reduced by approximately 10% in the presence of dimethyl sulfoxide which acts as a weak enzyme inhibitor.⁵⁾ A consecutive reaction was also observed in plasma as well as by the everted intestine.

From the results described above, the behavior of PETN in the gastrointestinal tract of rat is considered to be as follows: Orally administered PETN is not absorbed and hydrolyzed in the stomach. After transferring to the small intestine in the unchanged form, it considerably undergoes the hydrolysis in the intestinal mucosa by an ester-splitting enzyme. And the released nicotinic acid is rapidly absorbed.

A tendency was observed that the hydrolysis rate of the ester which had the smaller number of nicotinic acid was remarkably slower. It seems, therefore, probable that these ester-form hydrolysates are absorbed partly in their original forms and enzymatically hydrolyzed to release nicotinic

acid in the blood. In addition the very low solubility of PETN at the intestinal pH (35 $\mu\text{g}/\text{ml}$, pH 6.5) may be one of the important factors for the absorption and hydrolysis of the drug as suggested by Harthon and Brattsand.¹⁵⁾ Further studies are required to investigate the absorption behavior of each ester-form hydrolysates.

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FIG. 5. Hydrolysis Profile of PETN in Rat Plasma
 ○, PETN; ●, Tri; △, Di; ▲, nicotinic acid.
 Each point represents the mean of 3 experiments with S.D. The lines are drawn to fit the points as closely as possible.

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