STUDIES ON THE ABSORPTION OF SODIUM GAIAZULENE-3-SULFONATE. I*  

HIDEYA MUKAI, KATSUHIRO Sugihara, and MAKOTO Sugiyama  
Research Laboratories, Nippon Shinyaku Co., Ltd., Nishiohji Hachijo, Minami-ku, Kyoto, 601, Japan  
(Received August 30, 1984)  

From the viewpoint of safety and efficacy of sodium guaiazulene-3-sulfonate (GAS) after oral cavity administration, the absorption behavior of GAS was investigated in rats and rabbits and following results were obtained. 1) GAS was absorbed not from oral cavity but from nasal cavity in rats and rabbits. 2) The in situ perfusion experiments revealed the existence of dose-dependent specific absorption mechanism in the rat small intestine. 3) GAS was absorbed from neither the rat stomach nor the rectum. These facts reveal that GAS transfers into the systemic circulation only through the small intestine after the clinical application to the oral cavity. And these absorption characteristics of GAS are suited for its direct action on the inflamed oral mucosa. When GAS is administered into the oral cavity, the safety is at least the same as that obtained after oral administration.  

Keywords — sodium guaiazulene-3-sulfonate; oral cavity; nasal cavity; gastrointestinal tract; in situ perfusion; absorption; site specificity  

Sodium guaiazulene-3-sulfonate (GAS), the water-soluble derivative of guaiazulene, has been widely applied for the stomatitis, the pharyngitis and the gastritis clinically. The studies on the absorption of GAS were already reported by Fujimoto et al.1,2) They reported that the plasma concentration of GAS was higher after oral cavity administration than after oral administration. However, under their experimental conditions, the absorption site of GAS is not necessarily clarified to be the oral mucosa because the nasal cavity as well as the oral cavity is filled with GAS solution.  

In order to elucidate this problem, we studied the absorption behavior of GAS after oral cavity administration.  

MATERIALS AND METHODS  

Chemicals — GAS was supplied by Nippon Shinyaku Co., Ltd. All other chemicals were of reagent grade.  

Animals — Wistar male rats (200–250 g) and JW-Nibs male rabbits (2–3 kg) were used. Animals were fasted overnight prior to absorption experiments, but water was given ad libitum.  

In Vivo Experiments — 1) Intravenous and Oral Administration: GAS was administered orally or intravenously in a 8 mg/kg dose as a saline solution (2 mg/ml) to conscious animals. 2) Oral Cavity Administration: Animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) prior to surgery. After the insertion of polyethylene tube (2.67 mm outside diameter) into the trachea and the ligature of the esophagus, GAS was administered into the oral cavity in a 8 mg/kg dose as an aqueous solution (10 mg/ml) according to the method by Fujimoto et al.2,3) 3) Nasal Cavity Administration: GAS was administered to the nasal cavity through the nostril in a 2.2 mg/kg dose as an aqueous solution (10 mg/ml). The operation for the nasal absorption study was the same as that  

* Parts of this work were presented at the 102nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, 2, April 1982.
reported by Hirai et al. 4)

Blood samples were taken periodically from the femoral vein of rats and the marginal ear vein of rabbits.

In Situ Experiments — 1) Oral Mucosa Perfusion: The procedure and the apparatus were the same as that reported by Anno et al. 5) 2) Gastrointestinal Tract Perfusion (Stomach, Small Intestine and Rectum): The method by Kakemi et al. was employed. 6,7)

Each 0.2 ml of the sample solution was pipetted at given time after the perfusion.

Preparation of the Injured Oral Mucosa — To make the inflamed oral mucosa, acetic acid (25 μl, 5% in saline) was injected submucously according to the method of Ono et al. 8) The animals were used for the experiments at about 20 h after injection of acetic acid.

Pretreatment to Prevent the Drug Solution in the Oral Cavity from Flowing into the Nasal Cavity — Rats were treated as shown in Fig. 1. Rats anesthetized with sodium pentobarbital were incised on the neck to expose the trachea and the esophagus. After the insertion of polyethylene tube (2.67 mm outside diameter) into the trachea (e), it was inserted into the esophagus (f) toward the posterior part of the nasal cavity. The nasopalatine tract (c) was closed with an adhesive agent. After this treatment, the drug solution in the oral cavity could not flow into the nasal cavity.

Analytical Methods — GAS in plasma and perfusate was determined by high performance liquid chromatographic (HPLC) method, and detail operation and analytical conditions are shown in Chart 1. The concentration of GAS was calculated from the peak height ratio utilizing the calibration curve. Phenol red in perfusate was analyzed by the same method as GAS in perfusate. Fig. 2 shows the chromatograms of the plasma sample and the perfusate. The calibration curve of GAS concentration in plasma is shown in Fig. 3.

RESULTS

The time courses of plasma concentration after intravenous, oral and oral cavity administration of 8 mg/kg of GAS to rats and rabbits are shown in Fig. 4. The systemic availabilities after

plasma 0.5 ml
4M-trichloroacetic acid 100 μl
I.S. soln 0.5 ml (25 μg/ml) a)
distilled water 0.5 ml
shaking for 5 min
centrifuging for 10 min
20 μl of supernatant inject in HPLC

per fusate

↓
diluted with I.S.
soln (12.5 μg/ml) a)

↓
20 μl of mixture inject in HPLC

CHART 1. Determination Procedures of GAS in Plasma and Per fusate and HPLC Conditions

a) I.S. = Sodium anthraquinone-β-sulfonate
HPLC conditions

Apparatus: Model 6000A solvent delivery system, Model U6K universal injector, Model 440 ultraviolet detector (Waters Assoc., Milford, MA, U.S.A.).

Column: Nucleosil 10 SB (Macherey-Nagel, D-5160 Düren), pre-column: Bondapak C18 Corasil (Waters Assoc., Milford, MA, U.S.A.).

Mobil phase: 0.212% LiCl soln(methanol, acetoni trile, distilled water = 1:1:1).

Flow rate: 1 ml/min.
FIG. 2. High-Performance Liquid Chromatograms of GAS and I.S. in Plasma (I) and of GAS and Phenol red in Per fusate (II)
A: GAS, B: internal standard, C: GAS, D: phenol red.

FIG. 3. Calibration Curve of the Peak Height Ratio (GAS to I.S.) versus Plasma Concentration of GAS
Each point represents the mean ± S.D. (n=3). The curve was drawn by the least squares regression analysis.
\[ y = 0.0299x - 0.0210, r = 0.9996 \]

oral and oral cavity administration to rats calculated by the comparison of the area under the plasma concentration curve (0–7 h) with that after i.v. injection, were 21.6 and 18.2%, respectively. The corresponding values in rabbits were 25.4 and 47.2%, respectively. These results indicate that GAS, as well as some aminophenicillins\(^9\),\(^{10}\) and water-soluble dyes\(^{11,12}\) can be well absorbed from the gastrointestinal tract and the oral cavity in spite of being completely ionized form in all pH range.

To estimate the absorption when GAS is administered into the inflamed oral cavity, we applied it to rats and rabbits with the acet ic acid-induced inflamed oral mucosa. The time courses of GAS plasma concentration is shown in Fig. 5, comparing with the results after dosing to animals with the intact oral mucosa. As a result, there was no significant difference in plasma concentration between the damaged and intact animals. Consequently, it is found to be doubtful whether GAS in actually absorbed through the oral mucosa.

Then, in order to identify the real absorption site of GAS we performed in situ perfusion experiments on the intact and the damaged oral mucosa of rats and rabbits. In all cases, the GAS
FIG. 4. Plasma Concentration-Time Courses of GAS after Intravenous (–○–), Oral (–○–) and Oral Cavity (–△–) Administration of 8 mg/kg Dose
I: administration to rats, II: administration to rabbits. Results are expressed as the means ± S.D. of at least 4 animals.

FIG. 5. Plasma Concentration-Time Courses of GAS in Animals with Damaged Oral Mucosa after Oral Cavity Administration of 8 mg/kg Dose, Comparing with Those in Animals with Intact Oral Mucosa
I: rats (–○–) damaged, (–△–) intact. II: rabbits (–○–) damaged, (–△–) intact.
Results in animals with the intact oral mucosa are the same as those showed in Fig. 4 (–△–). Each point is expressed as the mean ± S.D. of 4 animals.
concentration in perfusate did not show any decrease within 2 h and the plasma concentration of GAS could not be detected. These facts indicate that GAS is not absorbed through the oral mucosa and the absorption after oral cavity administration showed in Fig. 4 can be due to the nasal cavity absorption because of the unintended flow of GAS solution into the nasal cavity of rats and rabbits with ligated esophagus.

In order to prove the presumption mentioned above, GAS plasma concentration was determined after nasal and oral cavity administration to rats pretreated to prevent the drug solution flowing into the nasal cavity according to the operation method in Fig. 1. As shown in Fig. 6, GAS was found to be absorbed very rapidly from nasal cavity but not to be absorbed from oral cavity itself.

On the basis of these findings concerning the site specificity of absorption, we measured also the absorption ratios of GAS from each site of the gastrointestinal tract.

First, we investigated the absorption of GAS from the stomach or the rectum using the in situ perfusion technique. GAS remained almost completely in the perfusate within 3 h and was not detectable in plasma. These results show that GAS is not absorbed from the stomach and the rectum.

Secondly, we investigated the absorption from the small intestine. As shown in Fig. 7, GAS disappeared from the perfusate following the apparent first-order kinetics and the absorption rate was dependent on the initial concentration of GAS in the perfusate. These results suggest that the absorption of GAS from the gastrointestinal tract has also site specificity, i.e. GAS is absorbed only from the small intestine according to specific transport mechanism (detailed in the second report).

To estimate the ability of absorption in vivo from the nasal cavity and the small intestine, the absorbed fraction of the dose and the absorption rate after oral and nasal cavity administration were determined by simultaneous fitting of

---

**FIG. 6.** Plasma Concentration-Time Courses of GAS in Rats Treated According to Fig. 1 after Nasal (○, 2.2 mg/kg) and Oral Cavity (△, 8 mg/kg) Administration

GAS could not be detected in plasma after oral cavity administration. Results are expressed as the means ± S.D. of at least 3 animals.

**FIG. 7.** Disappearance of GAS through the Small Intestinal Mucosa of Rats

Initial concentration (μg/ml) of GAS in perfusate: a) 1000, b) 100, c) 10.

Results are expressed as the means ± S.D. of at least 3 animals.
plasma data after intravenous, oral and nasal cavity dosing to the two-compartment open model. According to the compartment theory, the following equations were used,

1) i.v. injection

\[ C = \frac{X_0(\alpha - k_{21})}{V_c(\alpha - \beta)} \exp(-\alpha t) + \frac{X_0(k_{21} - \beta)}{V_c(\alpha - \beta)} \exp(-\beta t) \]

\[ \alpha + \beta = k_{12} + k_{21} + k_{10} \]

\[ \alpha \beta = k_{21} k_{10} \] \hspace{1cm} (1)

2) oral administration

\[ C = \frac{k_{a_1} F_2 X_0}{V_c} \left( \frac{k_{21} - k_{a_1}}{\alpha - k_{a_1}} \frac{\beta - k_{a_1}}{\beta - k_{a_1}} \right) \exp(-k_{a_1} t) + \frac{k_{21} - \alpha}{(k_{a_1} - \alpha)(\beta - \alpha)} \exp(-\alpha t) \]

\[ + \frac{k_{21} - \beta}{(k_{a_1} - \beta)(\alpha - \beta)} \exp(-\beta t) \] \hspace{1cm} (2)

3) nasal cavity administration

\[ C = \frac{k_{a_3} F_2 X_0}{V_c} \left[ \frac{k_{21} - k_{a_3}}{(\alpha - k_{a_3})(\beta - k_{a_3})} \exp(-k_{a_3} t) \right. \]

\[ + \frac{k_{21} - \alpha}{(k_{a_3} - \alpha)(\beta - \alpha)} \exp(-\alpha t) \]

\[ + \frac{k_{21} - \beta}{(k_{a_3} - \beta)(\alpha - \beta)} \exp(-\beta t) \] \hspace{1cm} (3)

where, \( C \) is the plasma concentration (concentration in central compartment), \( k_{12}, k_{21} \) and \( k_{10} \) are rate constants, \( X_0 \) is dose in mg, \( V_c \) is distribution volume of central compartment, \( F_1 \) and \( F_2 \) are the fractions of absorbed drug, \( k_{a_1} \) and \( k_{a_2} \) are the absorption rate constants. These pharmacokinetic parameters with the least square method are listed in Table I. Fig. 8 shows the observed and the model-predicted values of GAS plasma concentration after oral and nasal cavity administration. These results show that GAS is absorbed much more rapidly and efficiently from the nasal cavity than from small intestine.

<table>
<thead>
<tr>
<th>Parameter (dimension)</th>
<th>Value ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha ) (h(^{-1}))</td>
<td>7.26 ± 3.15</td>
</tr>
<tr>
<td>( \beta ) (h(^{-1}))</td>
<td>0.562 ± 0.08</td>
</tr>
<tr>
<td>( k_{21} ) (h(^{-1}))</td>
<td>3.03 ± 1.27</td>
</tr>
<tr>
<td>( V_c ) (ml·kg(^{-1}))</td>
<td>31.2 ± 4.5</td>
</tr>
<tr>
<td>( F_1 )</td>
<td>0.451 ± 0.078</td>
</tr>
<tr>
<td>( k_{a_1} ) (h(^{-1}))</td>
<td>0.177 ± 0.038</td>
</tr>
<tr>
<td>( F_2 )</td>
<td>0.635 ± 0.060</td>
</tr>
<tr>
<td>( k_{a_3} ) (h(^{-1}))</td>
<td>27.6 ± 9.7</td>
</tr>
<tr>
<td>( k_{10} ) (h(^{-1}))</td>
<td>1.35</td>
</tr>
<tr>
<td>( k_{12} ) (h(^{-1}))</td>
<td>3.44</td>
</tr>
</tbody>
</table>

* a) Model is given in the chart below.

Each parameter except \( k_{10} \) and \( k_{12} \) was estimated by a least square regression analysis. \( k_{10} \) and \( k_{12} \) were derived from the following equations.

\[ k_{10} = \frac{\alpha \beta}{k_{21}} , \quad k_{12} = \alpha + \beta - k_{12} - k_{10} \]

FIG. 8. Observed Points and Simulated Curves of GAS Plasma Concentration after Oral (— O —) and Nasal Cavity (— ● —) Administration

Curves represent model predictions calculated by two-compartment open model with parameter values of Table I.
DISCUSSION

It was reported by Fujimoto et al. that GAS was well absorbed from the oral cavity of rats and rabbits.\(^1\)\(^,\)\(^3\) Similar results were obtained in our experiments (Fig. 4). But, from the following results, it became evident that GAS plasma concentration after oral cavity administration to animals was due to the absorption not through the oral membrane but the nasal membrane by the unintended flow of drug solution into the nasal cavity.

1) In the perfusion experiments, GAS was not absorbed from the oral membrane. 2) GAS was rapidly absorbed from the nasal cavity. 3) By utilizing the rats treated to prevent the drug solution from flowing into the nasal cavity, GAS could not be detected in plasma after oral cavity administration.

Recently, the nasal cavity has attracted the interest as a good absorptive site.\(^1\)\(^3\) However, it may not be suited for a site of administration of GAS because it is expected to be directly effective on the inflamed site.

The in situ perfusion experiments with the gastrointestinal tract of rats showed that GAS was absorbed only from the small intestine according to dose-dependent transport mechanism. The absorption from the small intestine is much slower than that from the nasal cavity, suggesting differences of the absorption mechanisms between the two sites.

As to the absorption mechanisms of GAS, membrane components of the absorption sites may be concerned. The details about it will be reported in the succeeding paper.

The absorption characteristics of GAS described above favor the efficacy and the safety after the clinical application to the oral cavity. It is preferable to be retained in the inflamed oral cavity without the absorption through the oral membrane in consideration of the mechanism of the pharmacological effect of GAS, i.e. it has the direct action on the inflamed cells. The safety after oral cavity administration will be guaranteed to the level of that obtained after oral administration because the maximum plasma concentration of the former is presumably lower than that of the latter at the same clinical dose.

REFERENCES
