PHARMACOLOGICAL PROPERTIES OF A NEW ANTICHOLINERGIC AGENT, 1, 1-DIMETHYL-5-METHOXY-3-(DITHIEN-2-YLMETHYLENE) PIPERIDINIUM BROMIDE (SA-504)

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In the course of screening anticholinergic agents, tipepidine hibenzoate (Asverin®), a piperidine derivative with a tertiary ammonium nitrogen and a potent antitussive agent (1), was found to have a marked anticholinergic activity (2).

It is well known that among atropine and scopolamine derivatives, the anticholinergic activities of quaternary ammonium compounds are generally more potent than their tertiary derivatives (3). This also holds true with piperidine derivatives (2, 4). Therefore, efforts were made to search for an anticholinergic with strong spasmolytic activity among many quaternary ammonium derivatives of piperidine that would have minimal untoward effects, such as mydriasis and antisynergogue characteristics of most anticholinergics. As a result, a new compound, 1,1-dimethyl-5-methoxy-3-(dithien-2-ylmethylene) piperidinium bromide (SA-504), was selected for further studies (2) (Fig. 1).

![Fig. 1. Chemical structure of SA-504.](image)

The present paper describes anticholinergic and spasmolytic activity in vivo plus other pharmacological activities including acute toxicity in comparison with other known anticholinergic agents.

METHODS

Antispasmogenic activity on isolated smooth muscle

1) Isolated guinea pig ileum

Spasmolytic effects of SA-504, hyoscine-N-butylbromide (HB), prifinium bromide (PB) and atropine sulfate (Atr) on the isolated ileum of guinea pig was examined, using
methacholine, nicotine, histamine and BaCl₂ as spasmogens. The ileal strips were suspended in air-bubbled Tyrode's solution maintained at 25 ± 1°C, and the contractile responses recorded isotonically. The activity was assessed by recording cumulative responses to the spasmogens before and 5 min after drug addition, and expressed in terms of the pA₂ and pD'₂-values according to van Rossum (5).

2) Isolated rat uterus

Diesterus rats determined from the "vaginal smear test" were used. The isolated uterus was suspended in air-bubbled Lock-Ringer's solution kept at 26 ± 1°C. Methacholine and oxytocin were used as spasmogens and activities of the drugs were expressed in terms of the pA₂- and pD'₂-values (5).

Gastrointestinal motility

Rats, fasted for 24 hr before the experiments, were anesthetized with i.v. urethane (1 g/kg). Gastric motility was observed by recording the pressure change according to the balloon method as described by Perret et al. (6).

1) Stomach contraction induced by vagal stimulation

The tip of the balloon was fixed to the pyloric region of anesthetized rats through a midline abdominal incision. A uniform brief contraction of the stomach was induced by electrical stimulation (1–3 V, 2 msec duration, 10 per sec for 3 sec) of the cervical vagus at 2 min intervals. Drugs were injected into the cannulated femoral vein and inhibitory actions on the stomach contraction were examined.

2) Spontaneous motility of the stomach

Spontaneous motility of the stomach of anesthetized rats was recorded by the method described above. The motility was enhanced by bethanechol infusion at a constant rate of 0.5 mg/kg/hr, and then the drugs were injected into the femoral vein.

3) Gastrointestinal propulsion

Gastrointestinal propulsion was examined according to the method of Lauener et al. (7). Male mice were fasted for 18 hr before the experiments. Ten min after i.p. administration of the drugs, 0.2 ml of 5 per cent charcoal, suspended in 0.5 per cent carboxymethyl cellulose, was administered orally. Thirty min later, the animals were sacrificed by cervical fracture and the small intestine removed. Inhibition of traverse of the charcoal meal was determined by estimating the percentage in length of the part containing charcoal powder.

Gastric secretion

After fasting for 24 hr, the pylorus-ligated rats with acute fistula were prepared according to the method described by Ishii (8). Volume of the secreted gastric juice was measured at 30 min intervals and free and total acid contents were titrated with 0.1 N NaOH using Töpfer and phenolphthalein as indicators. Drugs were injected into the tail vein.

Anti-ulcerogenic effects

1) Ulcer formation in Shay rats

Pylorus-ligated rats were prepared according to the method described by Shay et al. (9). Drugs were administered s.c. immediately after the ligation. After twenty hr, the
rats were sacrificed under ether anaesthetic and the stomachs removed. Degree of ulceration was estimated according to the method of Takagi et al. (10), with a modified scoring system.

2) Ulcer formation in 'stress' rats

Rats held in stress cages were immersed in a water bath at 24°C to the depth of the xiphoid of the animals according to the method of Takagi et al. (11). The rats were administered the drugs s.c. 10 min before immersion. After seventeen hr the animals were sacrificed by decapitation, and the stomachs removed. Severity of ulceration of each affected area was scored as follows: weak hyperaemia 0.25, severe hyperaemia 0.5, weak hemorrhage 1 and severe hemorrhage 2. Areas of mucosal damage were measured in mm² using a planimeter, multiplied by the corresponding scores, and summed up to give an ulcer index.

Mydriatic activity

Mydriasis caused by the drugs was microscopically observed in male mice and rats. Drugs were administered i.p. in mice and i.v. in rats.

Antisialagogue activity

Groups of five rats were anesthetized with urethane (1 g/kg, i.p.) and salivation was induced by s.c. injection of 2 mg/kg of pilocarpine. Immediately after injection of pilocarpine, drugs were administered into the tail vein. Salivation was measured every 15 min by weighing a cotton pellet which had been inserted into the mouth.

In the experiment with mice, the drugs were given i.p. to groups of 5 mice. Twenty min later, the animals were administered 10 mg/kg of pilocarpine s.c. The inhibitory effect of the drugs was estimated from the number of mice which did not show any salivation within 20 min after administration of pilocarpine. The value of ED₅₀ was calculated by the method of Weil (12).

Ganglion blocking activity

The ganglion blocking activity of the drugs was examined with preparations of cervical sympathetic nerve-nictitating membrane in spinal cats according to Burn's method (13). The contraction of nictitating membrane was induced by electrical stimulation (1–3 V, 0.1 msec duration, 10 per sec for 3 sec) of the sympathetic preganglionic fiber at 3 min intervals and recorded by means of a strain gauge. Drugs were injected into the femoral vein.

Contraction of skeletal muscle

In rabbits anesthetized with sodium pentobarbital (30 mg/kg, i.p.), contraction of the extensor digitorum longus muscle was recorded by a strain gauge connected to the tendon end of the muscle. The contraction was induced by electrical stimulation (1 V, 1 msec duration, 1 per sec) of the peripheral end of the cut nerve. The drug was administered into the marginal vein of the ear.

Respiration and cardiovascular system

1) Blood pressure, heart rate and respiration

Male rats, dogs and cats were anesthetized by i.p. injection of 1 g/kg of urethane, 30 and 35 mg/kg of sodium pentobarbital respectively. Blood pressure was recorded
from the femoral artery with a pressure transducer. Heart rate was recorded by cardio
tachography triggered by the arterial pulse and respiratory rate was counted directly. The drug was injected into the femoral vein.

2) Electrocardiogram

Male guinea pigs were anesthetized with urethane (1.5 g/kg) by i.p. administration. ECG was recorded by the augmented unipolar extremity lead of the left limb. The drug was injected into the cannulated femoral vein.

3) Contraction of isolated heart

Isolated heart of guinea pig was prepared according to the method of Langendorff (14). The heart preparation was perfused with oxygenated Lock-Ringer's solution at 25°C. The drug solution (0.2 ml or less) was injected into the aortic cannula and the contraction was recorded by means of a strain gauge fixed at the apex of the heart.

Diuretic action

Rats were given saline solution (3 per cent of body wt) orally twice at an interval of one hr. Immediately after the 2nd administration of saline, the drug was given by the oral route (0.5 ml/100 g body wt). Urine excreted during a 5 hr period was collected and total volume determined. Potassium and sodium ions in the urine were measured by means of an Evans flame photometer.

Formation of methemoglobin

After i.p. administration of the drug to rats, blood was withdrawn from the tail vein at intervals of 30 min and subjected to spectrophotometry for methemoglobin according to the method of Evelyn et al. (15).

Local anesthesia and irritation

The drug was injected intracutaneously on the back of the guinea pig forming a wheal. Local anesthetic action was examined according to the method of Bulbring et al. (16). The number of pricks on the wheal failing to give the skin-squeezing response was counted and the value of ED₉₉ determined. Local irritation was tested according to the method of Miles (17). Evans-blue dissolved in saline was injected i.v. and the stained area of the wheal measured.

Acute toxicity

Seventy-two hr after administration of the drug to dd-strain mice and Sprague-Dawley rats, the value of LD₅₀ was calculated by the method of Weil (12).

RESULTS

Antispasmodic activity on isolated smooth muscle

1) Isolated guinea pig ileum

The antagonistic effects of SA-504 and other spasmolytic agents on the isotonic contraction of the isolated guinea pig ileum are shown in Table 1. Drugs antagonized the contraction induced by methacholine more than those by the other spasmogens. The PA₂-value of SA-504 against methacholine was 8.37 and almost equal to that of PB and
TABLE 1. Antagonistic activities of SA-504 and other anticholinergics on spasmodogen-induced contractions in isolated guinea pig ileum.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Methacholine</th>
<th>Nicotine</th>
<th>BaCl₂</th>
<th>Histamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pA₂</td>
<td>pA₂</td>
<td>pD₂</td>
<td>pA₂</td>
</tr>
<tr>
<td>SA-504</td>
<td>8.37±0.16</td>
<td>7.66±0.16</td>
<td>6.97±0.12</td>
<td>5.64±0.20</td>
</tr>
<tr>
<td></td>
<td>(1.0)</td>
<td>(1/5.5)</td>
<td>(1/540)</td>
<td>(1/220)</td>
</tr>
<tr>
<td>HB</td>
<td>7.09±0.13</td>
<td>6.93±0.14</td>
<td>5.57±0.13</td>
<td>5.80±0.09</td>
</tr>
<tr>
<td></td>
<td>(1.0)</td>
<td>(1/1.4)</td>
<td>(1/20)</td>
<td>(1/20)</td>
</tr>
<tr>
<td>PB</td>
<td>8.35±0.15</td>
<td>7.77±0.14</td>
<td>6.66±0.15</td>
<td>6.37±0.14</td>
</tr>
<tr>
<td></td>
<td>(1.0)</td>
<td>(1/3.8)</td>
<td>(1/96)</td>
<td>(1/25)</td>
</tr>
<tr>
<td>Atr</td>
<td>8.57±0.14</td>
<td>7.84±0.18</td>
<td>6.84±0.14</td>
<td>5.71±0.13</td>
</tr>
<tr>
<td></td>
<td>(1.0)</td>
<td>(1/5.4)</td>
<td>(1/720)</td>
<td>(1/1900)</td>
</tr>
</tbody>
</table>

The value is the mean±standard error of 8-12 experiments.
The value in the parentheses is relative potency ratio (anti-methacholine activity = 1.0).

Both pA₂ and pD₂ were calculated for the inhibition of nicotine-induced contraction since these drugs show partially competitive and partially non-competitive antagonism against nicotine.

2) Isolated rat uterus

Table 2 summarizes antagonistic activities of the drugs on the methacholine- and oxytocin-induced contractions of the isolated uterus. The antagonistic activity to methacholine was greater than that to oxytocin in each drug. SA-504 exhibited a far more potent blockade of the oxytocin-induced contraction than the other anticholinergics, and antagonistic action was partially non-competitive as was papaverine in higher doses.

Gastrointestinal motility

1) Effect on contraction of the stomach induced by vagal stimulation

As shown in Fig. 2, inhibition of the contractions induced by vagal stimulation in
the rat was more potent in SA-504 than in HB, PB and Atr. The ED$_{50}$ values of SA-504, PB, Atr and HB were 10, 17, 32 and 50 μg/kg respectively. At equipotent doses, Atr exhibited a much longer duration of the action than SA-504, HB and PB.

2) Effect on spontaneous motility of the stomach

Inhibitory effects of the drugs on gastric motility induced by bethanechol infusion in rats is shown in Fig. 3. A dose of 40 μg/kg of these drugs produced inhibitory responses, i.e., a fall of the tone and abolishment of the peristaltic movement. Duration of action of these drugs was similar to those in vagal stimulation as described above.

3) Effect on gastrointestinal propulsion

Results are shown in Table 3. The activity of SA-504 nearly equaled that of HB and somewhat less than PB. A significant effect was observed with Atr even at lower doses
A NEW ANTICHOLINERGIC AGENT (SA-504)

Fig. 3. Effects of SA-504 and other anticholinergics on betahanechol-induced motility of rat stomach.

Table 3. Effects of SA-504 and other anticholinergics on gastrointestinal propulsion in mice.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Doses (mg/kg i.p.)</th>
<th>Per cent traverse of charcoal meal</th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>59±2.0</td>
<td></td>
</tr>
<tr>
<td>SA-504</td>
<td>0.2</td>
<td>47±2.4</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>46±4.0</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>45±3.8</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>24±2.2</td>
<td>58</td>
</tr>
<tr>
<td>HB</td>
<td>0.2</td>
<td>57±3.9</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>43±3.9</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>40±4.0</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>27±2.3</td>
<td>54</td>
</tr>
<tr>
<td>PB</td>
<td>0.2</td>
<td>46±4.6</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>32±3.4</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>31±3.8</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>18±1.2</td>
<td>69</td>
</tr>
<tr>
<td>Atr</td>
<td>0.08</td>
<td>37±3.9</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>36±5.4</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>26±3.8</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>23±3.3</td>
<td>61</td>
</tr>
</tbody>
</table>

Value is the mean±standard error of ten mice.
FIG. 4. Inhibitory effects of SA-504 and other anticholinergics on gastric secretion in acute fistula rats. Each drug was administered in a dose of 200 μg/kg (i.v.) at 0 hr. Inhibitory percentage is given as the mean of 5 rats. — O — SA-504, — — — HB, — — — PB, — — — Atr.

TABLE 4. Effects of SA-504 and other anticholinergics on gastric ulcer formation in Shay rats (20 hr after pyloric ligation).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Doses (mg/kg s.c.)</th>
<th>No. of expts.</th>
<th>Number of ulcers (mean)</th>
<th>Degree of ulceration*</th>
<th>Ulcer index**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>Control</td>
<td>--</td>
<td>32</td>
<td>24.4</td>
<td>8.5</td>
<td>1.3</td>
</tr>
<tr>
<td>SA-504</td>
<td>3</td>
<td>12</td>
<td>16.0</td>
<td>6.6</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9</td>
<td>10.7</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HB</td>
<td>10</td>
<td>12</td>
<td>19.1</td>
<td>5.5</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>9</td>
<td>22.2</td>
<td>8.0</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>8</td>
<td>7.0</td>
<td>4.3</td>
<td>0.3</td>
</tr>
<tr>
<td>PB</td>
<td>1</td>
<td>9</td>
<td>23.0</td>
<td>6.9</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12</td>
<td>15.7</td>
<td>4.4</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Atr</td>
<td>1</td>
<td>9</td>
<td>22.1</td>
<td>5.7</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9</td>
<td>15.3</td>
<td>5.4</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8</td>
<td>6.3</td>
<td>0.7</td>
<td>0</td>
</tr>
</tbody>
</table>

Value of ulcer index is the mean ± standard error.

* I : 0.5-1 mm in diameter, II : 1-3 mm, III : 3-5 mm, IV : 5-10 mm and perforation

** Sum of the number of ulcers in each degree multiplied by respective ulcer score (I : 1, II : 3, III : 5, IV : 10).

as compared with the other drugs.

**Effect on gastric secretion**

The inhibitory effect of SA-504 and other drugs on gastric secretion was examined
utilizing pylorus-ligated rats with fistula. Time courses of inhibition of both gastric secretion and acid output of these drugs are shown in Fig. 4. The inhibitory effect of Atr was the strongest, followed by PB, SA-504 and HB in that decreasing order. The output of free acid almost paralleled the secretion of gastric juice with each drug. Although free acid concentration was significantly reduced by each drug, total acid concentration remained at the normal level.

**Anti-ulcerogenic effects**

1) *Shay rat*

As illustrated in Table 4, the protective action of the drugs against ulcer formation was shown to be dose-dependent. SA-504 completely inhibited the ulcer formation at a dose of 30 mg/kg. SA-504 was significantly more effective than HB, while Atr and PB were nearly equal to or somewhat more potent than SA-504.

2) *Stress ulcer*

As shown in Fig. 5, Atr was the most potent compound with the potency of SA-504 being almost the same as that of PB and HB.

**Mydriatic activity**

Doses required for half-maximum mydriasis in rats and mice are shown in Table 5. Mydriatic activities of both SA-504 and HB were less than one-tenth of Atr. Fig. 6 shows the time courses of mydriasis after i.v. injection of the test drugs to rats. The dose of 30 μg/kg of Atr corresponding to the dose of ED₉₀ for gastric spasmolysis caused a long-lasting and nearly maximum effect on the pupil of the eye. On the other hand, 100 μg/kg of SA-504 corresponding to a 10 times dose that of ED₉₀ for spasmolysis caused

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**Fig. 5.** Relationship between dose and percent reduction of ulcer index in 'stress' rats. ○ SA-504, □ HB, ▲ PB, ■ Atr. Each point represents the average per cent value of 10 rats.
TABLE 5. Mydriatic activities of SA-504 and other anticholinergics in mice and rats.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Rats (i.v.)</th>
<th>Mice (i.p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA-504</td>
<td>0.30 (0.12–0.70)**</td>
<td>2.5 (1.2–5.2)</td>
</tr>
<tr>
<td>HB</td>
<td>0.36 (0.15–0.83)</td>
<td>6.0 (2.7–12.6)</td>
</tr>
<tr>
<td>PB</td>
<td>0.10 (0.03–0.33)</td>
<td>1.1 (0.4–2.4)</td>
</tr>
<tr>
<td>Atr</td>
<td>0.02 (0.01–0.04)</td>
<td>0.2 (0.1–0.4)</td>
</tr>
</tbody>
</table>

* Doses (ED50) dilating the pupil by 50 per cent of its maximal diameter reached with each drug were determined.

** 95 per cent confidence limits.

Fig. 6. Time courses for mydriatic action in rats. The value is the mean ± standard error of 5 rats. Drugs were administered i.v. — SA-504 100 µg/kg, — HB 100 µg/kg, — PB 100 µg/kg, — Atr 30 µg/kg.

only a slight and temporary dilation of the pupil.

Antisialagogue activity

Pilocarpine-induced salivation in rats is shown in Fig. 7. Intravenous administration of 100 µg/kg of SA-504 did not significantly affect saliva secretion, while the same dose of Atr prevented it completely. There was no significant difference among SA-504, HB and PB.

Table 6 also shows the ED50 values of the drugs on the pilocarpine-induced salivation in mice, based on all-or-none response. The antagonistic action of SA-504 was significantly less than that of Atr and PB, while HB was almost equipotent to SA-504.

Ganglion blocking activity

The dose-response relationship in each drug regarding inhibition of the nictitating membrane contraction induced by electrical stimulation of the preganglionic fiber of cats is shown in Fig. 8. At a dose of 3 mg/kg, SA-504, PB and HB inhibited more than 50 per cent of the contraction of the nictitating membrane, which indicates the presence of a ganglion blocking activity.
TABLE 6. Inhibitory effects of SA-504 and other anticholinergics on pilocarpine-induced salivation in mice.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>ED50 (mg/kg i.p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA-504</td>
<td>15.6 (7.3-33.4)*</td>
</tr>
<tr>
<td>HB</td>
<td>19.4 (12.5-30.1)</td>
</tr>
<tr>
<td>PB</td>
<td>2.4 (1.4-4.1)</td>
</tr>
<tr>
<td>Atr</td>
<td>0.6 (0.4-1.1)</td>
</tr>
</tbody>
</table>

* 95 per cent confidence limits

**Effect on the contraction of skeletal muscle**

The effect of SA-504 on the contraction of extensor digitorum longus muscle induced by electrical stimulation of the peripheral end of the cut nerve was examined using rabbits. SA-504 showed no effect at i.v. doses of 1 to 3 mg/kg.

**Effects on the respiratory and cardiovascular systems**

1) **Blood pressure, heart rate and respiration**

Intravenous administration of 100 μg/kg of SA-504 produced no marked effect on
blood pressure and respiration of anesthetized dogs, however the heart rate was slightly increased. Three hundred \( \mu g/kg \) of SA-504 caused a temporary fall of about 40 mm Hg in blood pressure and an increase in heart rate by about 20 per cent of the control level. In larger doses, 1 mg/kg of SA-504 caused a fall of about 70 mm Hg in blood pressure accompanied by a slight increase in the respiratory rate, which returned to normal in about 10 min. On the other hand, the heart rate was increased to the same extent as in the case of 300 \( \mu g/kg \).

The effect on cats and rats were somewhat weaker than that on dogs.

2) **Effects on electrocardiogram**

When injected into the femoral vein, SA-504 exhibited no influence on the wave patterns of the ECG in anesthetized guinea pigs at doses up to 1 mg/kg, although an increase in heart rate and a fall in blood pressure were observed.

3) **Effect on contraction of the isolated heart**

Contraction of the isolated perfused guinea pig heart was not influenced either in contractile force or beating rate by SA-504 even at a dose as high as 100 \( \mu g/heart \).

**Diuretic action**

SA-504 administered orally to rats at doses of 2 to 50 mg/kg did not affect the volume of urine or the excretion of potassium and sodium ions during a 5 hr period. Although excretion of red-tinted urine was observed at higher doses of SA-504, it was not due to hematuria as confirmed by the benzidine test.

**Formation of methemoglobin**

Intraperitoneal administration of 30 mg/kg of SA-504 to rats induced no methemoglobin formation, whereas the same dose of sodium nitrate resulted in a severe methemoglobin formation.

**Local anesthesia and irritation**

Infiltration anesthesia and irritation were examined utilizing the wheal test in guinea pigs.

<table>
<thead>
<tr>
<th>Route</th>
<th>Mice</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Intravenous</td>
<td>12.1</td>
<td>14.0</td>
</tr>
<tr>
<td>(10.6 - 13.9)*</td>
<td>(11.4 - 17.2)</td>
<td>(9.5 - 11.9)</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>97.2</td>
<td>113.5</td>
</tr>
<tr>
<td>(79.7 - 118.6)</td>
<td>(96.6 - 133.5)</td>
<td>(43.8 - 69.3)</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>145</td>
<td>175</td>
</tr>
<tr>
<td>(119 - 177)</td>
<td>(146 - 210)</td>
<td>(254 - 471)</td>
</tr>
<tr>
<td>Oral</td>
<td>713</td>
<td>770</td>
</tr>
<tr>
<td>(585 - 870)</td>
<td>(675 - 878)</td>
<td>(1368 - 2004)</td>
</tr>
</tbody>
</table>

* 95 per cent confidence limits
The local anesthetic effect of SA-504 was approx. four times as potent as procaine. As for local irritant action, a 0.1 per cent solution of SA-504 injected intracutaneously produced no significant action but an irritant action was observed with a 1 per cent solution.

Acute toxicity

LD₅₀ values of SA-504 with 95 per cent confidence limits in mice and rats by different routes of administration are shown in Table 7. It was found that behavioral response of mice and rats to SA-504 was essentially the same regardless of species and routes of administration. At toxic doses, SA-504 caused mydriasis, a dry mouth and a decrease in spontaneous activity. When lethal doses were administered, animals died of respiratory failure, and occasionally tremor-like convulsions were observed. LD₅₀ values did not differ between males and females.

DISCUSSION

The results presented in this paper demonstrate that SA-504, a new piperidine derivative, possesses strong anticholinergic activity. The in vitro anti-methacholine activity of SA-504 was nearly equal to that of Atr, i.e., the pA₂ values of SA-504 and Atr were 8.37 and 8.57, respectively. SA-504 and Atr inhibited to the same extent the nicotine- or Ba⁺⁺-induced contraction of isolated ileum preparation. The anti-nicotinic actions of both SA-504 and Atr were competitive at low doses and non-competitive at high doses to the spasmogenic action of nicotine. It was further observed that the ratio of anti-nicotinic to anti-methacholine activity, as estimated by pA₂, was 1/5.5 for SA-504 and 1/5.4 for Atr. These results indicate that SA-504 is an anticholinergic having properties similar to those of Atr.

The fact that the pA₂ values of SA-504 and Atr against nicotine are rather close to those against methacholine may indicate that the blocking action of these compounds on the nicotine-induced contraction is not ascribable to the anti-nicotinic action but mainly due to the antimuscarinic action that inhibits the action of acetylcholine released from the nerve terminal as the result of ganglionic stimulation induced by nicotine. In fact, it was demonstrated that the blockade by SA-504 of the cervical sympathetic ganglion required much higher doses than those required for reduction of the gastrointestinal contraction induced by vagal stimulation. Therefore, it is inferred that the ganglionic blocking action of SA-504 is much weaker than its antimuscarinic activity.

These observations suggest that, as in the case of Atr, SA-504 affects primarily the muscarinic site.

In the in vivo experiments, SA-504 was also found to have properties qualitatively similar to those of Atr, but some quantitative differences were observed between the two compounds. The inhibitory action of SA-504 on the stomach contraction induced by vagal stimulation was approx. 3 times as strong as Atr, whereas the duration of the action of Atr was much longer than that of SA-504. Similarly, SA-504 produced a somewhat stronger but less long-lasting inhibitory effect than Atr on gastric motility induced by bethanechol infusion. The blocking action of SA-504 against gastric secretion and
ulcer formation was weaker than that of Atr. The mydriatic and antisialagogue activities of SA-504 were much less potent than Atr. Thus, it is interesting that the in vivo activity of SA-504, which is thought to be ascribed to the similar anticholinergic action of Atr in vitro, is different from that of Atr.

Just as in the case of papaverine, SA-504 in higher doses produced a non-competitive inhibitory effect on the oxytocin-induced contraction of isolated rat uterus. Atr, however, did not show such a non-competitive action even at high doses. To date, there is no evidence indicating that SA-504 possesses so-called papaverine-like relaxing action on smooth muscle, however this property could be responsible for the in vivo activity difference between the two compounds. Other possibilities that could explain the organ specificity of SA-504 or Atr may be the difference in the metabolic rate between the two compounds in a particular organ or the versatility of cholinergic receptors of target organs. Further experiments are required to elucidate these possibilities.

SUMMARY

Pharmacological activities of 1,1-dimethyl-5-methoxy-3-(dithien-2-ylmethylene) piperidinium bromide (SA-504), a new antispasmodic agent, were investigated.

The in vitro anticholinergic activity of SA-504 almost equaled that of Atr. In in vivo experiments, inhibition of stomach contractions by SA-504 induced by vagal stimulation in the rat was more marked than by Atr. The inhibition of the nictitating membrane contraction induced by stimulation of the preganglionic fiber of the cat was slight. From these results the effects of SA-504 appear attributable to a potent postganglionic parasympathetic blocking action (antimuscarinic action).

Gastric secretion and ulcer formation in 'stress' and Shay rats were prevented when SA-504 was administered.

The mydriatic and antisialagogue activities of SA-504 were weaker than the effects on gastric motility. SA-504 did not influence the respiratory and cardiovascular systems at doses inhibiting the gastric motility. Contraction of the skeletal muscle, methemoglobin formation in blood and diuretic action were not detected even at high doses of SA-504. On the other hand, local anesthetic action of SA-504 was approx. four times that of procaine. The acute toxicity of SA-504 in mice and rats was determined utilizing various routes of administration. Toxic doses of SA-504 showed mydriasis, a dry mouth and depression. The animals died of respiratory failure when administered lethal doses.

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