Effects of a New Antihypertensive Agent, SGB-1534, on Rat Platelet Aggregation

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Abstract—The present study was designed to examine the antiplatelet activity of SGB-1534, 3-[[2-[4-(o-methoxyphenyl)-1-piperazinyl]ethyl]-2,4(1H, 3H)-quinazolinedione monohydrochloride, compared with prazosin, ketanserin and aspirin. The equihypotensive doses of SGB-1534, prazosin and ketanserin were administered orally to rats; and 1 hr later, their effects on collagen-induced platelet aggregation, compared with those of aspirin, were examined under ex vivo conditions. The bleeding time was determined by using the tail transection method. SGB-1534 (10 mg/kg) as well as ketanserin (3 and 10 mg/kg) and aspirin (10 mg/kg) effectively inhibited the platelet aggregation; and in addition, they significantly prolonged bleeding times. Prazosin in doses of 10 and 30 mg/kg did not affect either the aggregation or bleeding times. Whereas 10^{-4} M aspirin significantly inhibited the production of malondialdehyde (MDA) in rat platelets, SGB-1534, prazosin and ketanserin even in considerably high concentrations (10^{-4} or 10^{-3} M) did not affect the MDA production and the cyclic AMP levels in the platelets. In isolated rat femoral arteries, SGB-1534, prazosin and ketanserin antagonized the contractile response to phenylephrine with pA2 values of approximately 10.06, 10.39 and 7.71, respectively. Also, SGB-1534 and ketanserin attenuated the contractile response to 5-hydroxytryptamine (5-HT) with pA2 values of 6.36 and 9.53, respectively, while prazosin had no antagonistic effects on 5-HT-induced contraction.

SGB-1534 is a recently synthesized phenylpiperazine derivative, displaying potent and long-lasting hypotensive effects in several hypertensive models of rats (1). This antihypertensive agent possesses a peripheral \(\alpha_1\)-adrenoceptor antagonistic action, comparable to prazosin (2), a known selective antagonist at \(\alpha_1\)-adrenoceptors (3), which may be primarily responsible for its blood pressure-lowering effect. The other effect attributable to SGB-1534 is a 5-HT2 antagonism (2). This drew our attention to the fact that 5-HT plays an important role in platelet aggregation (4, 5) and that ketanserin, a selective antagonist at 5-HT2 receptors (6), effectively inhibits the aggregation (7).

The present experiment, therefore, was undertaken to investigate the effect of SGB-1534 on rat platelet aggregation and to explore some mechanism of action. Prazosin, ketanserin and aspirin were included in the investigation as reference drugs.

Materials and Methods

Animals: Male Sprague-Dawley rats weighing 250–300 g were used, unless otherwise noted. The animals were fasted for about 12 hr before experiments, but had free access to water.

Blood pressure measurements: Spontaneously hypertensive male rats (SHRs) of the Kyoto Wistar origin, weighing about 300 g (13 weeks of age), from a commercial supplier (Japanese Charles River Laboratories) were utilized. Under pentobarbital-Na anesthesia (50 mg/kg, i.p.), each rat was surgically implanted with an indwelling...
polyethylene cannula, as previously described (1), to directly monitor blood pressure (SBP) and heart rate (HR). Prior to blood pressure measurements, the animals were fasted overnight, and then the SBP was measured with a Nihon Kohden pressure transducer (MPU-0.5) and the HR determined with a Nihon Kohden heart rate counter (AT-600G) under conscious conditions. Drug solutions were administered orally in a volume of 1.0 ml. All recordings were made on a chart with a Watanabe Linearorder (model WR-3101).

Platelet aggregation: One hour after oral applications of drugs or vehicle, blood from abdominal aortae of rats anesthetized with ether was collected into the test tubes containing 20 U/ml heparin sodium (ratio of anticoagulant/final sample volume=1:10). To get platelet-rich plasma (PRP), the blood was centrifuged at 1300 rpm (230xg) for 5 min. After PRP was separated, the remaining aliquot was centrifuged at 3000 rpm (1300xg) for 10 min, and platelet-poor plasma (PPP) was obtained. PRP was diluted with autologous PPP to a final platelet count of 4x 10^8 platelets/ml. A platelet counter (Coulter Electronics, model ZBI) was used to adjust the number of platelets in PRP. Aliquots of pooled PRP (200 μl) were transferred to 1 ml glass cuvettes (76x50 mm, Nikko Bioscience, Ltd.). Each sample was preincubated at 37°C for 2 min in a blood platelet aggregometer (NKK platelet aggregation tracer, model PAT-4M, SSR Engineering Co.) under continuous stirring at 1000 rpm, and then 20 μl collagen was added. The final concentration of collagen was 5 μg/ml. All samples were estimated by duplicate assay within 30 min. The concentration of collagen produced about 60% of maximal aggregation. The aggregation curve was monitored by means of the NKK platelet aggregometer and recorded at a chart speed of 0.7 cm/min on a pen recorder (U-826DS, Nippon Denshi Kagaku). Inhibition of platelet aggregation was assessed by comparing maximal optical transmission changes of PRP samples obtained from drug-treated groups with those from the 0.9% saline (vehicle)-treated group, and these data were expressed as percentage changes.

Tail bleeding time: The rats were placed in a plastic cylinder with several openings from one of which the animal's tail emerged. To avoid possible effects of anesthesia on platelet-blood vessel interplay and drugs, unanesthetized rats were used. The animals were maintained at room temperature (23±1°C). One hour after oral administration of drugs or vehicle, bleeding times were measured by making a free-hand full transection of the tail 1 mm from the tip with a disposable surgical blade (8). The rats' tails were placed in 25 ml isotonic 0.9% saline solution of pH 7.4 at 37°C immediately after injury. Bleeding times were measured from the moment the tail was incised until bleeding stopped completely (no rebleeding within 30 sec).

Malondialdehyde (MDA) formation by platelets: MDA formation by platelets, as an indicator for prostaglandin biosynthesis, was evaluated spectrofluorimetrically using a modification of the method of McMillan et al. (9). Briefly, 0.9 ml of PRP in the presence of 0.1 ml of drug solution or 0.9% saline solution, was challenged with 0.1 ml of 5 mg/ml arachidonate sodium. After 30 min at 37°C, the reaction was stopped by the addition of 1 ml of 30% trichloroacetic acid. After extraction, the thiobarbituric acid reaction for MDA was performed and the fluorescence at 532 nm was measured with a Beckman DU-8B spectrophotometer. Results are expressed as nmol MDA/4x10^8 platelets/30 min, estimated by a standard solution of 1,1,3,3,-tetraethoxypropane.

Determination of platelet cyclic AMP (c-AMP): The procedure was described elsewhere (10). Briefly, PRP collected from 10 rats was centrifuged at 3000 rpm (1300xg), at 4°C, for 10 min. The pellet was resuspended in 25 mM Tris-HCl buffer (pH 7.5) containing 139 mM NaCl, after being rinsed twice with the buffer solution. The suspension was used for the assay of c-AMP. The incubation mixture, in a final volume of 0.5 ml, contained 0.4 ml of the platelet suspension (10^9 platelets/ml) and 0.1 ml of drug or 0.9% saline solution. After 5 min incubation at 37°C, 0.25 ml of 30% trichloroacetic acid was added to the mixture. Precipitated proteins were removed by
centrifugation, and the supernatant solution was assayed for c-AMP. Cyclic AMP was radioimmunochemically determined with a c-AMP Assay Kit (Yamasa Shoyu Co., Choshi), according to the method of Honma et al. (11).

**Isolated blood vessels:** Ring segments (2–3 mm length) of rat femoral arteries were suspended under a load of 2 g in a 10 ml organ bath containing a modified Krebs-Henseleit bicarbonate solution composed of (in mM): NaCl, 125.0; KCl, 5.6; MgSO4, 1.2; KH2PO4, 1.2; CaCl2, 2.5; NaHCO3, 25.0; and glucose, 9.0. The bath was aerated thoroughly with a gas phase mixture of 95% O2-5% CO2. The pH of the gassed solution was 7.4 at 37°C. The muscle segments were connected to a Nihon Kohden force transducer (SB-IT) for continuous recording of isometric tension. Recordings were made on a Yokogawa ink-writing pen recorder (model 3066). Five preparations were run concurrently. The muscle segments were equilibrated for at least 3 hr, with washes every 30 min, before exposure to drugs. The antagonists and agonists were added to the 10 ml organ bath in a volume of 0.1 ml. Subsequent doses of agonists were increased by a factor of about 10, and were introduced when the response of the preceding ones had reached a steady level. Thus, a cumulative concentration-percentage maximal response curve to an agonist drug was constructed for each tissue. In the preliminary experiment, the curve for each agonist was constructed 3 times in the absence of any antagonists. It was confirmed that there were no significant differences between the corresponding values obtained from the second and third curves. Therefore, after the second concentration-response curve was constructed, the tissue was washed out repeatedly in the absence of antagonists and equilibrated for at least 1 hr. An antagonist was added to the organ bath, and 10 min later, the cumulative concentration-response curve to an agonist drug was constructed again for each tissue. All drug concentrations are expressed as final molar (M) concentrations in the bath solution. The negative logarithm of the molar concentration causing a 2-fold shift to the right of the concentration-response curve for the agonist (pA2) was determined according to a Schild analysis (12).

**Drugs:** The drugs used were: 3-[2-[4-(o-methoxyphenyl)-1-piperazinyl]ethyl]-2,4(1H, 3H)-quinoxalinedione monohydrchloride (SGB-1534) (Nagano, H., Takagi, M., Kubodera, N., Matsunaga, I., Nabata, H., Ohba, Y., Sakai, K., Hata, S. and Uchida, Y.: Eur. Pat. 89065 (Sept. 21, 1983)), prazosin hydrochloride (Hess, H.E. and Conn, G.: U.S. Pat. 3511836 (May 12, 1970)) and ketanserin hydrochloride (Vandenberk, J., Kennis, L.E.J., Marul, J.M.C. and Van Heertum, A.H.M.T.: Eur. Pat. 13612 (Jan. 7, 1980)) (all synthesized at the Chugai Institute); /-phenylephrine hydrochloride, aspirin, prostaglandin E1 (PGE1), 5-hydroxytryptamine creatinine sulphate (5-HT) and arachidonate sodium salt (from porcine liver approx. 90%) (all from Sigma, U.S.A.); collagen (Hormonchemie, München GMBH), and papaverine hydrochloride (Tokyo Kasei). The drugs were dissolved in or diluted with 0.9% saline solution to the desired concentration.

**Statistical analysis:** The data in the text are expressed as the mean±S.E. Student’s t-test for unpaired observations was used for statistical evaluation of the data; P values less than 0.05 were considered to be statistically significant.

**Results**

**Blood pressure determination:** In a single dose of 3 mg/kg, SGB-1534 caused a significant decrease of blood pressure with no changes in heart rate. The hypotensive response to SGB-1534 did not return to the predose level during the 7 hr-experimental period. Similar patterns of hypotension were observed with prazosin (3 mg/kg) and ketanserin (10 mg/kg) administered orally. However, ketanserin significantly decreased heart rate, while prazosin increased it. As disclosed in Fig. 1 and Table 1, the hypotensive effects of SGB-1534 (3 mg/kg) were nearly comparable to those of prazosin (3 mg/kg) and ketanserin (10 mg/kg) in magnitude and duration. Treatment with equivalent volumes of 0.9% saline solution produced no significant changes in blood pressure and heart rate from the preadminis-
Platelet aggregation: SGB-1534 (10 mg/kg) administered orally effectively inhibited collagen-induced platelet aggregation (Table 2). Ketanserin (3 and 10 mg/kg, p.o.) and aspirin (10 mg/kg, p.o.) also significantly prevented the aggregation, while prazosin (10 and 30 mg/kg, p.o.) failed to inhibit it.

Bleeding time: Oral administration of SGB-1534 (3 and 10 mg/kg) induced a significant prolongation of bleeding time. Ketanserin (10 mg/kg, p.o.) and aspirin (3 and 10 mg/kg, p.o.) also prolonged the bleeding time, whereas prazosin (10 and 30 mg/kg, p.o.) did not affect it (Table 3).

Malondialdehyde (MDA) formation: Aspirin (10^-4 and 10^-3 M), an inhibitor of the cyclooxygenase pathway of arachidonic acid oxygenation, significantly inhibited by 58.8% and 69.3%, respectively, MDA production from rat platelets stimulated with arachidonic acid. However, even in considerably high concentrations, SGB-1534, prazosin and ketanserin, unlike aspirin, did not significantly affect the generation of MDA (Table 4).

Cyclic AMP (c-AMP) levels in platelets: As shown in Table 5, 10^-7 M prostaglandin E1 (PGE1), a stimulator of adenylyl cyclase or 10^-4 M papaverine, an inhibitor of c-AMP phosphodiesterase significantly increased c-AMP levels in rat platelets. However, even in high concentrations, SGB-1534, prazosin and ketanserin did not significantly affect the c-AMP levels.

Isolated blood vessel: SGB-1534, prazosin and ketanserin, in the concentrations used in the present experiment, did not affect resting tension in the rat femoral artery. Phenylephrine or 5-HT added to the organ bath induced reproducible muscle contractions. Curves of cumulative concentration-response to phenylephrine (10^-8-10^-3 M, each concentration given until stabilization of the response) were obtained before and 10 min after the administration of SGB-1534 (10^-6 M), prazosin (10^-6 M) or ketanserin (10^-7 M). These drugs caused a parallel shift to the right of the concentration-

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### Table 1. Effects of SGB-1534, prazosin and ketanserin administered orally on mean systemic blood pressure (SBP) and heart rate (HR) in conscious SHRs

| Drugs       | Dose (mg/kg) | N  | SBP (mean)         | HR                  |
|-------------|--------------|----|-------------------|---------------------|
|             |              |    | Pretreatment value (mmHg) | Peak % decrease | Pretreatment value (beats/min) | Peak % change |
| 0.9% Saline |              | 5  | 165.5±5.0         |                     | 354.0±15.4            |                |
| SGB-1534    | 3            | 5* | 178.0±9.3         | 30.4±3.7            | 405.0±14.4            | -3.7±9.1      |
| Prazosin    | 3            | 5* | 171.0±8.7         | 31.7±4.6            | 340.0±5.8             | +17.0±4.2     |
| Ketanserin  | 10           | 5  | 187.0±10.8        | 29.7±5.9            | 346.0±15.4            | -20.9±3.5     |

Each value represents the mean±S.E. of 5 observations on 5 rats, *except for HR responses to SGB-1534 and prazosin (each N=4).
response curve to phenylephrine. (Fig. 2) As shown in Table 6, the potency of SGB-1534 in antagonizing the contractile response to phenylephrine was nearly the same as prazosin, and it was approximately 220 times higher than that of ketanserin. Similarly, curves of cumulative concentration-response to 5-HT (10^{-8}–10^{-4} M)

| Table 2. Effects of SGB-1534, prazosin, ketanserin and aspirin administered orally on collagen-induced aggregation in rats |
|---------------------------------------------------------------|
| **Drugs**         | **Dose (mg/kg)** | **N** | **% Transmission** | **% Inhibition** |
|-------------------|-----------------|-------|-------------------|-----------------|
| 0.9% Saline       | 3               | 9     | 44.9±4.9          |                 |
| SGB-1534          | 10              | 5     | 34.6±7.1          | 22.9            |
| Prazosin          | 10              | 5     | 5.2±3.4**         | 88.4            |
| Ketanserin        | 30              | 5     | 43.6±2.8          | 2.9             |
| Aspirin           | 10              | 5     | 57.6±8.3          | -28.3           |
|                   | 3               | 5     | 4.2±5.1           |                 |
|                   | 10              | 5     | 28.8±4.1*         | 35.9            |

Drugs were administered orally 1 hr before the blood collection. Each value represents the mean±S.E. *P<0.05, **P<0.01, compared to the values obtained from the 0.9% saline-treated group.

| Table 3. Effects of SGB-1534, prazosin, ketanserin and aspirin administered orally on tail bleeding time in rats |
|---------------------------------------------------------------|
| **Drugs**         | **Dose (mg/kg)** | **N** | **Bleeding time (sec)** | **% of control** |
|-------------------|-----------------|-------|-------------------------|-----------------|
| 0.9% Saline       | 1               | 10    | 290.1±40.4              | 85.0            |
| SGB-1534          | 3               | 10    | 248.6±83.4              |                 |
|                   | 10              | 5     | 509.6±123.3*           | 175.7           |
| Prazosin          | 10              | 5     | 625.4±135.2**          | 215.6           |
| Ketanserin        | 3               | 5     | 433.4±117.9            | 149.4           |
|                   | 30              | 5     | 387.4±28.0             | 133.5           |
| Aspirin           | 10              | 5     | 416.4±196.4            | 143.5           |
|                   | 30              | 5     | 476.4±58.6*            | 164.2           |

Drugs were administered orally 1 hr before the measurement of bleeding time by the transection method. Each value represents the mean±S.E. *P<0.05, **P<0.01, compared to the values obtained from the 0.9% saline-treated group.

| Table 4. Effects of SGB-1534, prazosin, ketanserin and aspirin on malondialdehyde (MDA) production in rat platelets |
|---------------------------------------------------------------|
| **Drugs**         | **Concentration (M)** | **N** | **MDA (nmol/4×10^8 platelets/30 min)** | **% Inhibition** |
|-------------------|-----------------------|-------|----------------------------------------|-----------------|
| 0.9% Saline       | 10^{-4}               | 8     | 4.27±0.42                              |                 |
| SGB-1534          | 10^{-3}               | 4     | 3.60±0.04                              | 15.7            |
|                   | 10^{-4}               | 4     | 3.65±0.18                              | 14.5            |
| Prazosin          | 10^{-4}               | 4     | 3.54±0.06                              | 17.1            |
| Ketanserin        | 10^{-4}               | 4     | 4.50±0.10                              | -5.4            |
| Aspirin           | 10^{-4}               | 4     | 1.76±0.05**                            | 58.8            |
|                   | 10^{-3}               | 4     | 1.31±0.02**                            | 69.3            |

*Each value represents the mean±S.E. **P<0.01, compared to the values obtained from the 0.9% saline-treated group.
Table 5. Effects of SGB-1534, prazosin, ketanserin prostaglandin E₁ (PGE₁) and papaverine on cyclic AMP levels in rat platelets

| Drugs       | Concentration (M) | N  | Cyclic AMP (pmol/10⁶ platelets) |
|-------------|-------------------|----|---------------------------------|
| 0.9% Saline |                   | 3  | 1.02±0.31                       |
| SGB-1534    | 10⁻⁵              | 3  | 0.92±0.23                       |
|             | 10⁻⁴              | 3  | 0.97±0.25                       |
|             | 10⁻³              | 3  | 1.00±0.30                       |
|             | 10⁻²              | 3  | 0.91±0.22                       |
| Prazosin    | 10⁻⁶              | 3  | 1.00±0.24                       |
|             | 10⁻⁵              | 3  | 0.94±0.28                       |
|             | 10⁻⁴              | 3  | 0.93±0.27                       |
| Ketanserin  | 10⁻⁶              | 3  | 1.04±0.34                       |
|             | 10⁻⁵              | 3  | 1.06±0.35                       |
|             | 10⁻⁴              | 3  | 0.96±0.28                       |
| PGE₁        | 10⁻⁷              | 3  | 3.09±0.50*                      |
| Papaverine  | 10⁻⁴              | 3  | 3.26±0.47*                      |

Each value represents the mean±S.E.  *P<0.05, compared to the values obtained from the 0.9% saline-treated group.

Discussion

The present investigation indicated that SGB-1534 administered orally to rats, like ketanserin and aspirin, significantly inhibited collagen-induced platelet aggregations, and it prolonged bleeding times. Collagen seems to induce platelet aggregation mainly through two pathways: one involves the conversion of arachidonate in the platelet to prostaglandin (PG) intermediates (e.g., PGH₂, PGG₂), further being metabolized to the highly potent platelet aggregating substance TxA₂. The other is the platelet release reaction where the proaggregatory agents, e.g., ADP, epinephrine, norepinephrine and 5-HT are secreted (13, 14).

According to the present study, the mechanism of the antiplatelet actions of SGB-1534 and ketanserin seems to differ essentially from that of aspirin. While aspirin inhibited concentration-dependently the production of malondialdehyde (MDA), which is one of the stable breakdown products in the arachidonic pathway, SGB-1534 and ketanserin scarcely affected the production. Furthermore, it was evidenced that SGB-1534 did not significantly affect the curve (Fig. 2). As demonstrated in Table 6, the potency of SGB-1534 in antagonizing the contractile response to 5-HT was approximately 1500 times lower than ketanserin.
Table 6. \( pA_2 \) values of antagonistic effects of SGB-1534, prazosin and ketanserin to phenylephrine or 5-hydroxytryptamine (5-HT) in rat femoral artery segment preparations

| Drugs     | \( pA_2 \) values to phenylephrine | \( pA_2 \) values to 5-HT |
|-----------|-----------------------------------|------------------------|
| SGB-1534  | 10.06±0.15 (5)                     | 6.36±0.06 (5)          |
| Prazosin   | 10.39±0.07 (5)                     | <5 (5)                 |
| Ketanserin | 7.71±0.19 (5)                      | 9.55±0.11 (5)          |

Each value represents the mean±S.E. The number of experiments is shown in parentheses.

and ketanserin, unlike papaverine or PGE\(_1\), did not affect the c-AMP levels in rat platelets. On the basis of these findings, it is concluded that the platelet aggregation inhibitory effects of SGB-1534 and ketanserin are not ascribable to prostaglandin synthesis and c-AMP metabolism in platelets.

According to our previous studies (2), SGB-1534 possesses a selective antagonistic activity at peripheral \( \alpha_1 \)-adrenoceptors, comparable to prazosin, a known \( \alpha_1 \)-adrenoceptor antagonist (3). This was confirmed by our present experiments carried out in isolated femoral arteries of rats. Nevertheless, the inhibitory effect of SGB-1534 on the platelet aggregation cannot be ascribed to the \( \alpha_1 \)-adrenoceptor blocking activity, since the equipotentiative doses of prazosin failed to inhibit the collagen-induced platelet aggregation, and in addition, prazosin failed to prolong the bleeding time.

In the previous (2) and present experiments, it has been noted that there are some differences in the pharmacological profiles between SGB-1534 and prazosin. Unlike prazosin, SGB-1534 possesses a selective 5-HT\(_2\) receptor antagonistic activity, even though it was considerably weaker compared with that of ketanserin, a known 5-HT\(_2\) receptor antagonist (6). As well-known, 5-HT itself induces platelet shape changes and reversible aggregation (4, 5, 15). Additionally, the monoamine largely amplifies the human platelet aggregation response to various agonists including ADP, epinephrine, norepinephrine and collagen (4, 5, 15) and induces itself strong aggregation of human platelets, pre-sensitized with norepinephrine (15). Recently, De Clerck and Van Nueten (7) reported that ketanserin inhibits the reversible aggregation induced by 5-HT in human platelet-rich plasma; and they reported that after both in vitro and oral administration to man, it also reduces the serotonergic amplification of agonists involving norepinephrine. Thus, there have been many reports concerning a close interaction particularly between norepinephrine and 5-HT in platelet aggregation.

Taking these together into consideration, it is not surprising that in the present study ketanserin administered orally to rats effectively inhibited the collagen-induced platelet aggregation. However, the question remains as to how SGB-1534, compared with ketanserin, inhibits to the same extent the aggregation. So, it might be of value, here again, to note the investigation by Ball et al. (15). According to their finding, phen tolamine, a classical \( \alpha \)-adrenoceptor antagonist, inhibited norepinephrine-induced aggregation of human platelets while having little effect on 5-HT, even though potentiation of 5-HT responses by norepinephrine was blocked. Furthermore, BW501C67, a 5-HT antagonist, had little inhibitory effect on norepinephrine, while it blocked both normal and norepinephrine-potentiated 5-HT responses. Thus, the question may be resolved if it can be taken into consideration that a close interaction between 5-HT and norepinephrine plays an important role, e.g., increase in platelet sensitivity, in the collagen-induced platelet aggregation and that the adrenergic and serotonergic blocking activities of SGB-1534 have some influence on the interaction, leading to the effect of antiplatelet aggregation. However, the precise mechanism of the antiplatelet effects of SGB-1534 requires further elucidation.

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