Alpha-adrenoceptor antagonists and chemical sympathectomy exacerbate anaphylaxis-induced hypotension, but not portal hypertension, in anesthetized rats

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Alpha-adrenoceptor antagonists and chemical sympathectomy exacerbate anaphylaxis-induced hypotension, but not portal hypertension, in anesthetized rats. Anaphylactic shock is sometimes life-threatening, and it is accompanied by hepatic sympathetic activation; exacerbation of anaphylactic shock; hepatic sympathetic activation; hepatic sympathetic nerve activity; portal hypertension; hepatic sympathetic nerve activity; chemical sympathectomy; prazosin; phentolamine; hepatic sympathetic nerve activity.

from the sympathetic nerve terminals in anaphylactic shock have not been determined, although those of β-adrenoceptors and epinephrine released from the adrenal gland have been reported (32). It remains unknown whether the peripherally released norepinephrine exerts beneficial actions against anaphylactic shock; the effects of norepinephrine on the aforementioned pharmacological interventions and surgical hepatic sympathectomy, as well as the response of the efferent hepatic sympathetic nerve activity to anaphylactic hypotension of chemical sympathectomy has not been determined.

Hepatic venoconstriction, as observed consistently in experimental anaphylaxis models (7, 11, 13), plays a significant role in the genesis of anaphylactic hypotension: anaphylactic hepatic venoconstriction produces acute portal hypertension, which then causes splanchic blood pooling and plasma extravasation due to increases in both the capillary pressure and vascular permeability, resulting in decreased circulating blood volume, and finally anaphylactic hypotension. Hepatic venoconstriction in rats is caused by anaphylaxis-induced release of venoconstrictive mediators, such as leukotrienes, platelet-activating factor, and cyclooxygenase metabolites (4, 5). As an alternative mechanism, activation of the sympathetic nervous system may evoke anaphylactic hepatic venoconstriction; electrical stimulation of the rat hepatic sympathetic nerve induced constriction of the hepatic vessel (3, 9). Anaphylactic hypotension of chemical sympathectomy has not been determined.

Therefore, there were two major purposes in this study regarding anaphylactic systemic hypotension and portal hypertension: The first was to determine the effects on anaphylactic systemic hypotension of chemical sympathectomy with 6-OHDA and pharmacological blockade of α-adrenoceptors in anesthetized rats. The second was to determine the effects on anaphylactic portal hypertension of the aforementioned pharmacological interventions and surgical hepatic sympathectomy, as well as the response of the efferent hepatic sympathetic nerve activity to anaphylaxis in anesthetized rats.

MATERIALS AND METHODS

Animals. The experiments conducted in the present study were approved by the Animal Research Committee of Kanazawa Medical University. Sixty-nine male Sprague-Dawley (SD) rats (Japan SLC, Shizuka, Japan) weighing 362 ± 3 g were used in this study. Rats...
were maintained at 23°C under pathogen-free conditions on a 12:12-h dark-light cycle, and allowed food and water ad libitum.

**Sensitization.** Rats were sensitized by the subcutaneous injection of an emulsion made by mixing equal volumes of complete Freund’s adjuvant (0.5 ml) with 1 mg ovalbumin (grade V, Sigma) dissolved in physiological saline (0.5 ml) (23). Nonsensitized rats were injected with complete Freund’s adjuvant and ovalbumin-free saline.

**Chemical sympathectomy.** Sensitized rats (n = 6) and nonsensitized rats (n = 6) for the 6-OHDA group and the 6-OHDA nonsensitized group, respectively, were inapritoneionally administered of 6-OHDA dissolved in sterile saline supplemented with 0.01% ascorbic acid three times at a dose of 40 mg/kg (1 ml) on day 1, and 80 mg/kg (1 ml) on days 2 and 3. The experiments with 6-OHDA-treated animals were performed at 3 days after the last injection. This procedure produces a highly effective sympathetic denervation (8,18).

**Surgical preparation and recording hemodynamic variables.** Rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed supinely on a thermostatically controlled heating pad (ATC-101B; Unique Medical, Japan) that maintained body temperature at 36–37°C throughout the experiment. The adequacy of anesthesia was monitored by the stability of blood pressure and respiration under control conditions and during a pinch of the hindpaw. Supplemental doses of anesthetic (10% of the initial dose) were given inapritoneionally, as necessary. The trachea was intubated to facilitate spontaneous breathing. The right jugular vein was catheterized with a polyethylene tube (ID 0.4 mm, OD 0.6 mm) for measurement of the central venous pressure at expiration (CVP). The right femoral artery and vein were also catheterized with a polyethylene tube (ID 0.3 mm, OD 0.5 mm) for measurement of the mean systemic arterial pressure (SAP) and continuous infusion of saline (10 ml kg⁻¹ h⁻¹), respectively. Following a midline incision of the abdominal wall, a 24-gauge polyethylene catheter (Terumo, Tokyo, Japan) was inserted into the portal vein, for continuous measurement of the mean portal venous pressure (PVP). Except for the experiments of measurement of the hepatic sympathetic nerve activity, a pulsed Doppler flow probe (MC2PSB; Transonic Systems, Ithaca, NY) was placed on the portal vein for continuous measurement of the mean portal venous blood flow (PBF). In the hepatic sympathectomy group, denervation was accomplished by cutting all visible nerves along the hepatic artery and by stripping the connective tissue passing next to and along the course of the hepatic artery. Immediately thereafter, the hepatic artery was surrounded with cotton swabs previously soaked in 10% (vol/vol) phenol diluted in absolute ethanol.

The SAP, CVP, and PVP were continuously measured with pressure transducers (TP-400T; Nihon-Kohden, Tokyo, Japan), and the reference level was set at the level of right atrium. Heart rate (HR) was measured by triggering the SAP. These hemodynamic variables along with PBF were digitally displayed and recorded at 40 Hz by PowerLab (AD Instruments, Castle Hill, Australia).

The vascular resistances of the splanchic vascular beds (Rspl) and the portal vein (Rpv) were calculated as follows: Rspl = (SAP – PVP)/PBF and Rpv = (PVP – CVP)/PBF.

**α-Adrenoceptor blockade and sympathectomy studies.** In addition to the chemical sympathectomy (6-OHDA)-pretreated sensitized rats (6-OHDA group) (n = 6) and nonsensitized (6-OHDA nonsensitized group) rats (n = 6), sensitized rats were assigned to the control (nontreatment) group (n = 6) and the following pretreatment groups: 1) α₁-adrenoceptor antagonist prazosin (n = 6), 2) nonselective α-adrenoceptor antagonist phenolamine (n = 6), and 3) hepatic sympathectomy (n = 6). The nonsensitized group was also studied with the assignment of nonsensitized rats (n = 6).

**Statistics.** All results are expressed as the means ± SE. Comparison of individual values within a group was made by the repeated-measures ANOVA followed by the Bonferroni post hoc test. Comparison of individual points between the five and two groups was made by one-way ANOVA followed by Bonferroni post hoc test and Student’s t-test, respectively. Differences were considered statistically significant at P < 0.05.

**RESULTS**

**α-Adrenoceptor blockade and sympathectomy studies.** Table 1 shows the effects of the α-adrenoceptor antagonists, and chemical and hepatic sympathectomy on the basal levels of the variables. After pretreatment with prazosin or phenolamine, SAP decreased significantly, while PVP, CVP, and Rpv tended to decrease but not significantly. In the 6-OHDA groups, Rspl was significantly smaller than in the control group.

Figure 1A shows representative recordings of the variables from the control group, and Fig. 2 shows the summarized data. An injection of the antigen caused systemic hypotension and portal hypertension in the control group: SAP decreased by...
The basal levels of the variables before and after administration of α-adrenoceptor antagonists or saline

**Table 1. The basal levels of the variables before and after administration of α-adrenoceptor antagonists or saline**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (n = 6)</th>
<th>Prazosin (n = 6)</th>
<th>Phentolamine (n = 6)</th>
<th>6-OHDA nonsensitized (n = 6)</th>
<th>6-OHDA (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>After</td>
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<tr>
<td>Sympathetic nerve activity</td>
<td></td>
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<tr>
<td>Peripheral arterial resistance, mmHg</td>
<td>12.1 ± 0.6</td>
<td>12.2 ± 0.6</td>
<td>12.7 ± 0.2</td>
<td>12.3 ± 0.2</td>
<td>12.3 ± 0.2</td>
</tr>
<tr>
<td>Central venous pressure, mmHg</td>
<td>9.7 ± 0.7</td>
<td>9.9 ± 0.7</td>
<td>9.6 ± 0.5</td>
<td>9.1 ± 0.5</td>
<td>9.2 ± 0.5</td>
</tr>
<tr>
<td>Mean portal venous resistance, mmHg</td>
<td>5.5 ± 0.4</td>
<td>5.5 ± 0.4</td>
<td>5.3 ± 0.3</td>
<td>4.9 ± 0.3</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>Portal venous resistance, mmHg</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>Mean blood flow, ml/min</td>
<td>24.6 ± 1.6</td>
<td>24.6 ± 1.6</td>
<td>24.6 ± 1.6</td>
<td>24.6 ± 1.6</td>
<td>24.6 ± 1.6</td>
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<tr>
<td>Spatiohepatic resistance, mmHg</td>
<td>4.6 ± 0.3</td>
<td>4.6 ± 0.3</td>
<td>4.6 ± 0.3</td>
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<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>Portal venous resistance, mmHg</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.3</td>
</tr>
</tbody>
</table>

Note: Values are expressed as means ± SE. In the control, nonsensitized, 6-OHDA nonsensitized, and hepatic sympathectomy groups, saline was injected instead of prazosin or phentolamine. There were no significant differences between the control groups and the other groups, except the experiments of hepatic sympathectomy groups throughout the experimental period (Fig. 2E). There were significant differences in Rspl in the control group at 10–60 min after antigen, when PVP of the control group was increased significantly from the baseline. In contrast to the significant increase in Rspl of the control group, there was no significant increase in Rspl observed in the α-adrenoceptor antagonist and chemical sympathectomy groups throughout the experimental period (Fig. 2E).

Neither CVP nor HR significantly changed after antigen injection in any groups studied. The nonsensitized group showed no significant changes in the variables throughout the experimental period (Fig. 2). None of the animals in this study died during the experimental period of 60 min after antigen administration.

**Surgical hepatic sympathectomy study.** Figure 2 also shows the results of the hepatic sympathectomy group. Hepatic sympathectomy group showed responses similar to those in the control group. There were no significant differences in variables between these two groups.

**Hepatic sympathetic nerve activity study.** Normal responses of hepatic sympathetic nerve activity to a transient hypotension induced by an intravenous injection of SNP (30 μg/kg) were observed before challenging the antigen: the hepatic sympathetic nerve activity reflexively increased in response to SAP fall (136 ± 8% at −20 mmHg, 180 ± 19% at −40 mmHg). Figures 3 and 4 show the results of changes in the hepatic sympathetic nerve activity, along with SAP and PVP, after antigen challenge. The responses of SAP and PVP to the antigen in the sensitized rats in which hepatic sympathetic nerve activity was measured were similar to those of the control group for the other studies (Figs. 3A and 4, A and B).
Immediately after antigen, hepatic sympathetic nerve activity tended to decrease but not significantly (91 ± 2% of the baseline at 1.5 min; $P = 0.087$) in the presence of the SAP fall ($-37 ± 5$ mmHg at 1.5 min). Thereafter, the nerve activity remained unchanged for 60 min (Figs. 3A and 4C).

**Plasma catecholamine concentrations.** To confirm the effectiveness of chemical sympathectomy with 6-OHDA and the possible roles of norepinephrine released from the sympathetic nerve endings in anaphylactic hypotension, the plasma levels of norepinephrine and epinephrine were measured in the separate experiments. As shown in Fig. 5A, at baseline the norepinephrine levels in the 6-OHDA pretreated rats (55 ± 9 pg/ml) were significantly smaller by 56% than that of the control sensitized rats (125 ± 3 pg/ml). At 30 and 60 min after antigen injection, the 6-OHDA-pretreated rats showed a much smaller norepinephrine (322 ± 23 and 500 ± 87 pg/ml, respectively) than the control sensitized rats (423 ± 8 and 947 ± 130 pg/ml, respectively). In contrast, the epinephrine levels at 30 min after antigen injection were significantly higher in the 6-OHDA pretreated rats (3,691 ± 484 pg/ml) than in the control sensitized rats without 6-OHDA (1,604 ± 72 pg/ml) (Fig. 5B). Figure 5C shows the blood pressure responses of these rats, which were essentially the same as those of the rats without blood sampling as described above: SAP in the 6-OHDA-pretreated rats was significantly smaller than that of the control sensitized rats at 40–60 min after antigen injection.

**DISCUSSION**

We obtained three major findings: 1) chemical sympathectomy and α-adrenoceptor blockade inhibited the recovery from systemic hypotension with inhibition of the late increase in Rspl; 2) neither chemical, nor hepatic sympathectomy, nor α-adrenoceptor blockade attenuated anaphylaxis-induced hepatic portal hypertension, i.e., increase in PVP; and 3) the hepatic sympathetic nerve activity did not increase in the presence of SAP fall after antigen. These findings suggest that norepinephrine released from the sympathetic nerve endings does not play a compensatory role in the initial fall of SAP but facilitates its recovery due to vasoconstriction and that hepatic sympathetic nerve is not involved in anaphylaxis-associated hepatic vеноconstriction in anesthetized rats.

To the best of our knowledge, this is the first study to determine the effects of chemical sympathectomy and α-adrenoceptor blockade on experimental anaphylactic shock models. We clearly showed that these interventions did not augment the severity of anaphylactic hypotension but attenuated recovery from hypotension. Furthermore, this elimination of recovery due to chemical sympathectomy and α-adrenoceptor blockade may be caused by attenuation of delayed vasoconstriction because Rspl, as an indicator of the mesenteric and celiac arterial tone, did not increase at the late phase of 10–60 min in the chemical sympathectomy and α-adrenoceptor antagonist groups (Fig. 2E).

Notably, the rats pretreated with either 6-OHDA, phentolamine, or prazosin survived longer than 60 min after antigen in the present study. This finding contrasts with the results of our previous studies using the same anaphylaxis rat models (32, 33), in which all rats pretreated with the β2-adrenoceptor antagonists ICI118,551 or propranolol, died within 50 min after antigen; 40% of those pretreated with the β1-adrenoceptor antagonist atenolol died within 60 min. These results suggest that the detrimental action of α-adrenoceptor inhibition is less than that of β-adrenoceptor inhibition in anaphylactic hypotension. One of the explanations for this predominance of β-adrenoceptor is that β2-adrenergic receptor stimulation is essential for preventing protein extravasation across the microvascular walls in various states with enhanced vascular permeability (19, 28). This difference may explain why the clinical reports that described the aggravation of anaphylactic shock in patients administered α-adrenoceptor antagonists are limited (30) compared with those focusing on β-adrenoceptor antagonists (16).

As expected, in the 6-OHDA-pretreated rats, basal norepinephrine level was as low as 44% of that in control sensitized rats. In addition, the increase of norepinephrine after antigen was much smaller in the 6-OHDA-pretreated rats than in the intact sensitized rats (Fig. 5). We assume that the norepineph-

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**Fig. 1.** Representative recordings of the systemic arterial pressure, central venous pressure, heart rate, portal venous pressure, and portal venous blood flow after an intravenous injection of the antigen of ovalbumin (0.6 mg) in a control rat (A), and a prazosin-pretreated rat (B).
rine at baseline and post-antigen in the 6-OHDA-pretreated rats was released from the adrenal glands. Actually, the higher levels of epinephrine, which is released exclusively from the adrenal glands, at postantigen in the 6-OHDA rats than those in the control rats suggest the facilitated activation of the adrenal glands to release catecholamine of norepinephrine and epinephrine in compensation for the absence of norepinephrine release from the sympathetic nerve endings in the former rats. These findings indicate that the chemical sympathectomy was effectively performed in the present study.

The finding that surgical hepatic sympathectomy did not attenuate anaphylactic portal hypertension suggests that the hepatic sympathetic nerves are not involved in anaphylactic hepatic venoconstriction. Furthermore, the lack of attenuation by either chemical sympathectomy or the pharmacological α-adrenoceptor blockade reinforces the latter assumption. Finally, the absence of excitation of the hepatic sympathetic nerve activity supports this conclusion.

In the same rat anaphylaxis model as used in this study, the plasma level of norepinephrine increased 4-fold at 2.5 min

Fig. 2. Summary of the changes in the systemic arterial pressure (A), portal venous pressure (B), portal venous blood flow (C), portal venous resistance (D), and splanchnic vascular resistance (E) after an injection of the ovalbumin antigen. ●, control group (n = 6); □, prazosin group (n = 6); ⌂, phentolamine group (n = 6); ○, 6-OHDA group (n = 6); ◊, hepatic sympathectomy group (n = 6); ●, nonsensitized group (n = 6); ●, 6-OHDA nonsensitized group (n = 6). Values are expressed as means ± SE. *P < 0.05 vs. baseline; each point of variables for the α-adrenoceptor antagonist and chemical sympathectomy groups during the time indicated by the black bar in Fig. 2, A–D is significantly different from the corresponding baseline value except for the nonsensitized and 6-OHDA nonsensitized groups. #P < 0.05 vs. the control group. Each point of variables during the time indicated by the white bar in A and E is significantly different from the corresponding value in the control group except for the hepatic sympathectomy group.
Thus, we suppose that the circulating noradrenaline released from organs other than liver may contribute to constriction of the hepatic vessels, even if hepatic sympathetic nerve activity does not increase. Nonetheless, portal hypertension was not attenuated by either prazosin or phentolamine. One explanation is that the circulating levels of epinephrine, which could dilate hepatic vessels via $\alpha_2$-adrenoceptors (10, 25) increased much more than that of norepinephrine (32), counteracting the vasoconstrictor action of norepinephrine. However, this seems unlikely because $\alpha_2$-adrenoceptor blockade did not affect the anaphylaxis-induced increases in PVP of anesthetized rats (32, 33).

Here, for the first time, we demonstrated that hepatic sympathetic nerve activity did not increase, but tended to decrease, in response to anaphylactic hypotension. The absence of activation of the hepatic sympathetic nerve activity contrasts with the anaphylactic activation of renal sympathetic nerve activity in the previous study for rats (20). This indicates that the regional difference in sympathetic outflow (12) may exist between kidney and liver during systemic anaphylaxis of anesthetized rats. On the other hand, the present result of no significant change in hepatic sympathetic nerve activity is not surprising because renal sympathetic nerve activity did not increase in the presence of SAP fall during anaphylaxis in dogs (15). It is likely that the baroreceptor reflex may not operate normally during anaphylactic hypotension. Further studies are required in this respect.

The mechanism for the hepatic venoconstriction during anaphylaxis of the present study, other than hepatic sympathoexcitation, may be related to vasoactive chemical mediators released in response to the antigen. We previously reported using isolated blood-perfused rat livers sensitized with ovalbumin that cysteinyl leukotrienes and cyclooxygenase products are mainly involved in anaphylactic hepatic venoconstriction (5). Buxton et al. (4) also reported that the cyclooxygenase products and platelet-activating factor are responsible for the antigen-induced venoconstriction of crystalloid-perfused livers from rats sensitized with BSA.

As a limitation of this study, the anesthesia of pentobarbital sodium might have substantial effects on the results. Pentobarbital decreases baroreceptor reflex control of both HR and renal sympathetic nerve activity in rats (26). Indeed, in the present study, HR did not significantly increase when SAP decreased after the antigen. The absence of tachycardia during anaphylactic hypotension of the pentobarbital-anesthetized SD rat was consistent with the previous studies reported by ourselves (6, 23, 24) and by others (20), and this finding contrasts with the tachycardia response of the unanesthetized SD rats that were sensitized and induced anaphylaxis in the same manner as in the present study (31). Furthermore, compared with the anesthetized anaphylactic rat, the sensitized unanesthetized rat recovered more quickly from the anaphylactic hypotension (31). In this respect, pentobarbital sodium might have exerted direct depressant effects on the myocardium and systemic vasodilating action (1, 27), which could potentiate the circulatory effects of anaphylaxis and provide more serious reactions in this setting.

In summary, we determined the roles of norepinephrine and $\alpha$-adrenoceptor in anaphylaxis-induced systemic hypotension and portal hypertension in anesthetized ovalbumin-sensitized SD rats. We used pharmacological procedures to destroy the sympathetic nerve endings, and to inhibit $\alpha$-adrenoceptors, as well as the surgical hepatic sympathectomy. We also measured directly the efferent hepatic sympathetic nerve activity. We found that $\alpha$-adrenoceptor antagonists and chemical sympathectomy exacerbate anaphylaxis-induced hypotension only at the late phase, but not survival. Anaphylactic portal hypertension was not affected by any perturbations studied. In conclusion, norepinephrine released from the sympathetic nerve endings does not play a compensatory role in anaphylactic hypotension at the early phase but does at the recovery phase, presumably, via vasoconstriction. Hepatic sympathetic nerve activity is not involved in the antigen-induced hepatic venoconstriction during anaphylactic hypotension in anesthetized rats.
Perspectives and Significance

The hemodynamic regulation in anaphylactic shock has not been fully understood. Although the knowledge on the defense system against hemorrhagic shock is well established, that against anaphylactic shock is not well known. Activation of the sympathetic nervous system is crucial against hemorrhagic shock. The present study showed that the norepinephrine released from the sympathetic nerve endings and activation of α2-adrenergic receptors exerted beneficial effects on anaphylactic hypotension at the recovery phase in rats. However, the sympathetic response to anaphylactic shock seems to be different depending on species: In response to the fall in SAP after antigen injection, the renal sympathetic nerve activity increases in rats (Ref. 20 and unpublished observation), whereas it does not necessarily increase in dogs (15). In addition, the present study has demonstrated that the hepatic sympathetic nerve activity did not significantly change when SAP substantially decreased after antigen injection in anesthetized rats. This finding indicates the presence of regional difference in sympathetic response to anaphylactic hypotension. Thus, clarification is needed to demonstrate further the mechanism for the central regulation of hemodynamics during anaphylactic shock. In addition, the roles of other compensatory mechanisms, such as the ANG II and vasopressin system in anaphylactic shock remains unknown, although the patients administered ANG II synthesis inhibitors or its receptor antagonists show increased

Fig. 4. Summary of the changes in the systemic arterial pressure (A), portal venous pressure (B), and hepatic sympathetic nerve activity (C) after an injection of the ovalbumin antigen. ●, the sensitized rats (n = 5); ▲, the nonsensitized rats (n = 6). Values are expressed as means ± SE. *P < 0.05 vs. baseline. #P < 0.05 vs. the nonsensitized rats.

Fig. 5. Summary of the plasma norepinephrine levels (A), plasma epinephrine levels (B), and mean systemic arterial pressure (C) after an injection of the ovalbumin antigen. ■, the 6-OHDA pretreated rats (n = 5); ●, the control-sensitized rats (n = 5); ▲, the nonsensitized rats (n = 5). Values are expressed as means ± SE. *P < 0.05 vs. baseline; #P < 0.05 vs. the sensitized rats.
severity of anaphylactic shock (21). There is a possibility that fatal outcome of anaphylactic shock might be due to impairment of these defense systems. Future studies in this respect are required.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: M.W. and M.T. performed experiments; M.W. and M.T. analyzed data; M.T., T.S., and Y.K. interpreted results of experiments; M.T., T.S., and Y.K. drafted manuscript; M.T. prepared figures; M.T., T.S., and Y.K. conceived and designed the research.

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