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ORIGINAL ARTICLE

The Effect of Adenosine A2A Receptor Antagonist Istradefylline on Multiple Organ Dysfunction in Heatstroke Rats

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Background: Global warming increases the incidence of heatstroke, which is the most severe heat illness. The mortality in patients with heatstroke is due to neurological disability and multiple organ failure caused by systemic inflammatory response. Adenosine A2A receptor antagonist has both neuroprotective and anti-inflammatory effects. Thus, we examined whether a new A2A receptor antagonist istradefylline has beneficial effects in heatstroke rats. **Methods:** Wistar rats were divided into four groups: (1) control group, with vehicle only (intravenous [iv] for 10 min); (2) control + istradefylline group, with 0.3 mg/kg istradefylline (iv for 10 min); (3) heatstroke group, rectal temperature reached 44.1°C, and then returned to room temperature with vehicle (iv for 10 min); and (4) heatstroke + istradefylline group, rectal temperature reached 44.1°C, and then returned to room temperature with 0.3 mg/kg istradefylline (iv for 10 min). During the experimental period, rectal temperature, heart rate, blood pressure, and pressor responses to norepinephrine (NE) were monitored. Before and after the rats were put into heating chamber, and after the rats returned to room temperature for 6 h, their blood was taken to analyze creatine kinase, lactate dehydrogenase, blood urea nitrogen, creatinine, alanine transaminase, albumin, total protein, and platelet count. In addition, the blood flow of tongue, left limb, and right limb was also monitored. Finally, we examined their survival rate. **Results:** In the present study, heatstroke rats showed high core body temperature accompanied with cardiac abnormalities and multiple organ dysfunction, mimicking the clinical manifestations of heatstroke patients. Treatment of heatstroke rats with istradefylline only partially improved platelet loss and vascular hyporeactivity to NE. However, istradefylline had no significant effects on cardiac abnormalities and multiple organ dysfunction in rats with heatstroke. **Conclusions:** These results suggest that although istradefylline has a mild impact on abnormal platelet count and pressor response to NE in heatstroke, both effects are unlikely to counteract multiple organ dysfunction and the mortality.

Key words: Heatstroke, A2A receptor, istradefylline, multiple organ dysfunction

INTRODUCTION

Rising global temperature in the past few decades is the major risk factor leading to heatstroke. Heatstroke is a lethal illness characterized by high core temperature owing to the failure of thermoregulatory capacity.[1,2] Moreover, thermoregulatory disturbances cause exaggerated acute response and severe elevation in core temperature, contributing to multiple organ dysfunction.[3] Heatstroke has been regarded as the most fatal critical disorder related to natural disasters due to its hospital mortality rate up to 62.6%.[4,5] Thus, searching for new therapeutic approaches that can effectively improve the outcome of heatstroke is crucial.

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Early intensive care unit (ICU) mortality in patients with heatstroke is thought to be due to multiple organ failure caused by systemic inflammatory response.[1,6,7] With continuous hyperthermia, direct heat-related cytotoxicity and acute physiological changes result in the dysregulation of inflammatory reaction.[7-9] Much like sepsis, systemic inflammatory response can ultimately lead to disseminated intravascular coagulation, multiple organ dysfunction, and death. In addition, the central nervous system is extremely sensitive to hyperthermia, and hence, neurological and cognitive dysfunction is one of the serious complications in heatstroke.[5,10] Previous studies show that late ICU mortality in patients with heat-related disorders is largely due to neurological disability.[6,11] Therefore, seeking for effective drugs that can counteract neurological disability and systemic inflammatory response in heatstroke is urged.

Recent findings suggest that adenosine A2A receptor is a putative therapeutic target for neurological and inflammatory disorders. The blockade of adenosine A2A receptors not only improves motor performance but also reverses cognitive dysfunction in Parkinson's disease.[12,13] In addition, inactivation of adenosine A2A receptor diminishes pro-inflammatory cytokine levels induced by middle cerebral artery occlusion or polymicrobial sepsis.[13,14] These findings indicate that adenosine A2A receptor antagonist has neuroprotective and anti-inflammatory effects. Istradefylline, a selective adenosine A2A receptor antagonist, shows (i) significant motor improvement in Parkinson's disease and has been used in clinical,[15,16] (ii) has been shown to improve cognitive function and memory problem in aging mice with amyloid pathology,[17] and (iii) can decrease the activation of immune cells and the production of inflammatory cytokines.[14,18,19] Thus, we hypothesized that adenosine A2A receptor might contribute to heatstroke-induced neurological disability and systemic inflammatory response, contributing to multiple organ dysfunction and even death. To address this hypothesis, we investigated the effects of istradefylline on heatstroke-induced multiple organ dysfunction in rats.

METHODS

Animals and experimental protocols

This study was approved by the Institutional Animal Care and Use Committee of National Defense Medical Center (Taipei, R. O. C., Taiwan). All experiments followed the National Institutes of Health guidelines for the treatment of animals and ethical animal research. Male Wistar rats (10–12 weeks old) were obtained from BioLASCO Taiwan Co (Taipei, Taiwan). The left carotid artery and right jugular vein of rats were cannulated for hemodynamic detection and drug administration. After recovering from the cannulation, the animals were induced to heatstroke by putting in a heating chamber of 42°C (with relative humidity of 40%–60%) till the rectal temperature reached 44.1°C, followed by 0.3 mg/kg istradefylline or vehicle administration (iv for 10 min) and observed for 6 h. The changes of core temperature, hemodynamics (i.e., mean arterial pressure, heart rate, and pressor response to 1 μg/kg norepinephrine [NE]), and blood flow were measured during the experimental period. In addition, the blood samples were collected to analyze the alterations of muscle injury index (i.e., creatine kinase [CPK] and lactate dehydrogenase [LDH]), kidney function index (i.e., blood urea nitrogen [BUN] and creatinine [CRE]), liver function index (i.e., alanine aminotransferase [ALT], albumin [ALB], and total protein [TP]), and platelet number.

Recording of core temperature and hemodynamic parameters

The core temperature was examined by placing a digital thermometer (VT-801; Valeo Inc., New Taipei City, ROC, Taiwan) into the rectum of the rats at baseline (i.e., time – 1 h) and specified times (i.e., 0, 1, 2, 4, and 6 h after removing from the heating chamber). The mean arterial pressure, heart rate, and the pressor response to NE (1 μg/kg) were also monitored by a pressure transducer (P23ID, Statham, Oxnard, CA, USA) and exhibited on a polygraph recorder (MacLab/4e, ADInstruments, Castle Hill, Australia).

Detection of organ injury and function

The blood samples were obtained at baseline (i.e., time – 1 h) and specified times (i.e., 0 and 6 h after removing from the heating chamber). The serum was used to investigate muscle injury index (i.e., CPK and LDH), renal function index (i.e., BUN and CRE), and hepatic function index (i.e., ALT, ALB, and TP) by Fuji DRI-CHEM 3030 (Fuji Photo Film, Tokyo, Japan). Each volume of blood drawn was replenished by injecting an equal volume of normal saline.

Assessment of platelet count

The blood samples were collected in sodium citrate tubes and the platelet number was examined by using an automatic cell counter (Sysmex KX-21N Hematology Analyzer; Sysmex America Inc., Mundelein, IL, USA).

Measurement of blood flow

The rats were anesthetized with sodium pentobarbital at baseline (i.e., time – 1 h) and specified times (i.e., 0 and 6 h after removing from the heating chamber) in order to

measure the blood flow in tongue, left limb, and right limb by laser speckle contrast imager (Moor Instruments, Devon, UK).

Statistical analysis

All results were shown as mean ± standard error of mean of *n* determinations, where *n* means the number of rats studied. Statistical significance between the groups was evaluated by two-way analysis of variance followed by Newman–Keuls test. $P < 0.05$ was statistically significant.

RESULTS

Changes of core temperature and hemodynamic parameters in heatstroke rats treated with istradefylline

The basal core temperature and hemodynamic parameters were not significantly different among all groups. After the rectal temperature reached 44.1°C in both heatstroke and heatstroke + istradefylline groups, they were removed from the heating chamber. It is noted that there were no significant differences in rectal temperature among all groups during the 1 h to 6 h observation [Figure 1a]. The heat stress caused a significant increase in heart rate at time 0–6 h after removing the rats from the heating chamber. Moreover, there were no significant differences in heart rate between heatstroke and heatstroke + istradefylline groups [Figure 1b]. In addition, heat stress caused a significant decrease in the mean arterial pressure at 1 h and pressor response to NE at time 0–6 h after removing the rats from the heating chamber [Figure 1c and d]. However, the treatment of heatstroke rats with istradefylline partially, but significantly, improved vascular hyporesponsiveness at 4 h after heat stress [Figure 1d].

Changes of muscle injury and renal function in heatstroke rats treated with istradefylline

The basal serum levels of CPK, LDH, BUN, and CRE were not significantly different among all groups. Serum levels of CPK, LDH, BUN, and CRE significantly increased at 6 h after heat stress [Figure 2a-d]. However, all these functional indexes were not significantly different between heatstroke and heatstroke + istradefylline groups [Figure 2a-d].

Changes of hepatic function in heatstroke rats treated with istradefylline

The basal serum levels of ALT, ALB, and TP were not significantly different among all the groups. Serum ALT levels significantly increased, and serum ALB and TP levels significantly diminished at 6 h after heat stress [Figure 3a-c]. However, all the liver functional indexes were not significantly different between heatstroke and heatstroke + istradefylline groups [Figure 3a-c].

Changes of peripheral blood flow in heatstroke rats treated with istradefylline

The basal blood flow of tongue, left limb, and right limb was not significantly different among all the groups. The heat stress caused significant increases in the blood flow of the tongue, left limb, and right limb at 0 h after removing the rats from the heating chamber [Figure 4 a-c]. However, there were no significant differences in peripheral blood flow between heatstroke and heatstroke + istradefylline groups [Figure 4a-c].

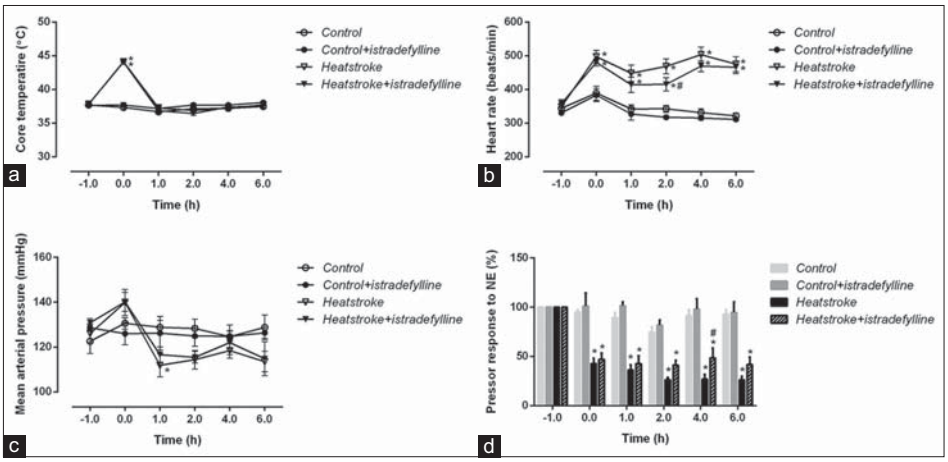

Figure 1: Effects of istradefylline on (a) core temperature, (b) heart rate, (c) mean arterial pressure, and (d) pressor response to norepinephrine in heatstroke rats. Depicted are the changes of core temperature and hemodynamics in animals that received vehicle (control, $n = 6$), received istradefylline (control + istradefylline, $n = 4$), heatstroke plus vehicle (heatstroke, $n = 6$), and heatstroke plus istradefylline (heatstroke + istradefylline, $n = 7$). Data are expressed as mean ± standard error of mean * $P < 0.05$, all versus control; $^{\#}P < 0.05$, with versus without istradefylline in heatstroke rats

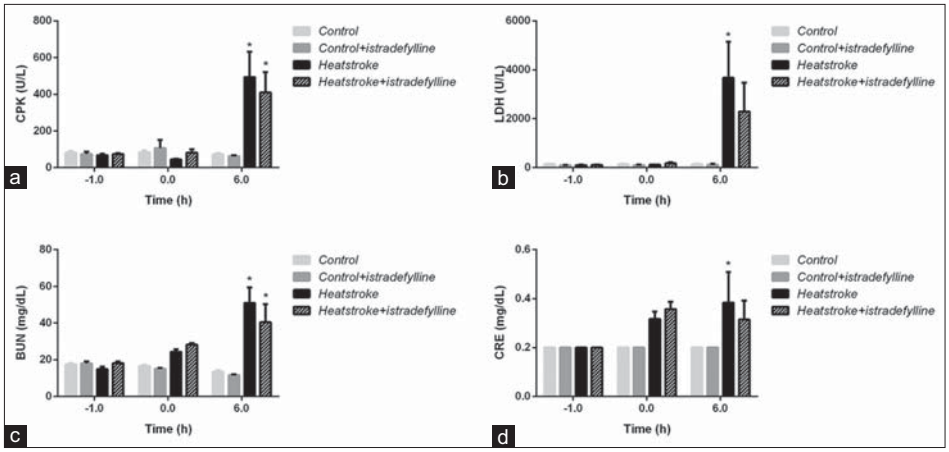

Figure 2: Effects of istradefylline on (a) creatine kinase, (b) lactate dehydrogenase, (c) blood urea nitrogen, and (d) creatinine in heatstroke rats. Depicted are the changes of muscle injury and renal function in animals that received vehicle (control, $n = 6$), received istradefylline (control + istradefylline, $n = 4$), heatstroke plus vehicle (heatstroke, $n = 6$), and heatstroke plus istradefylline (heatstroke + istradefylline, $n = 7$). Data are expressed as mean ± standard error of mean * $P < 0.05$, all versus control

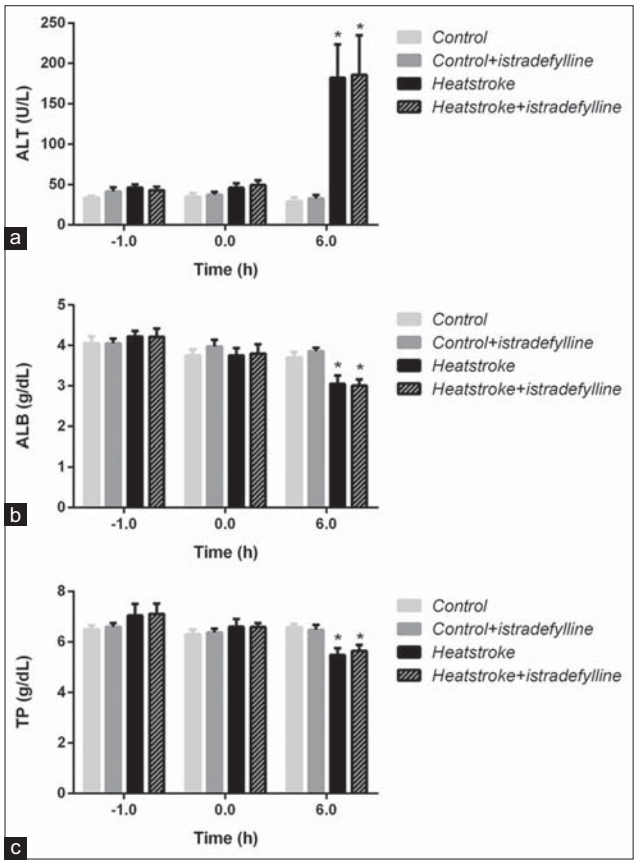

Figure 3: Effects of istradefylline on (a) alanine aminotransferase, (b) albumin, and (c) total protein in heatstroke rats. Depicted are the changes of hepatic function in animals that received vehicle (control, $n = 6$), received istradefylline (control + istradefylline, $n = 4$), heatstroke plus vehicle (heatstroke, $n = 6$), and heatstroke plus istradefylline (heatstroke + istradefylline, $n = 7$). Data are expressed as mean ± standard error of mean * $P < 0.05$, all versus control

Changes of platelet number in heatstroke rats treated with istradefylline

The basal platelet number was not significantly different among all the groups. The platelet count significantly reduced at 6 h after removing the rats from the heating chamber [Figure 5]. However, the treatment of heatstroke rats with istradefylline significantly ameliorated platelet loss at 6 h after heat stress [Figure 5].

Changes of survival rate in heatstroke rats treated with istradefylline

No mortality was observed within 6 h in both control and control + istradefylline groups [Figure 6]. The 6-h survival rates of heatstroke and heatstroke + istradefylline groups were 67% and 78%, respectively [Figure 6], showing no significant differences in 6-h survival rates between these two groups [Figure 6].

DISCUSSION

Previous studies suggest that adenosine A2A receptor antagonist is a therapeutic target for neurological and inflammatory disorders.[12-14] In addition, istradefylline has been shown to improve cognitive function in aging mice with amyloid pathology and attenuate the activation of immune cells as well as the production of inflammatory cytokines.[14,17-19] Thus, we investigated the therapeutic effects of istradefylline on heatstroke rats in this study. An extreme elevation in core temperature is the most obvious sign of patients with heatstroke. Rise in skin blood flow is one of the primary heat exchange mechanisms to reduce core temperature and protect against heat injury. In this study, when the rectal temperature

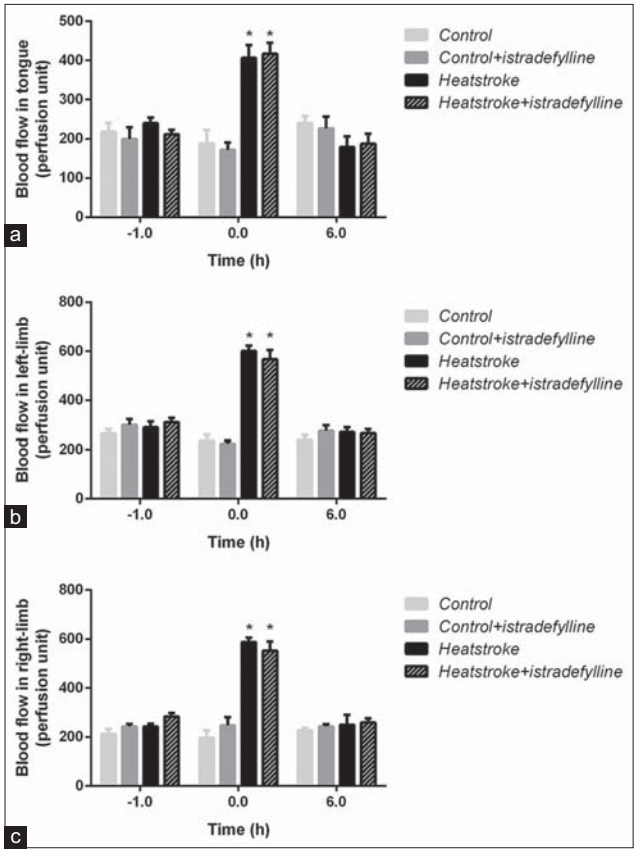

Figure 4: Effects of istradefylline on blood flow of (a) tongue, (b) left limb, and (c) right limb in heatstroke rats. Depicted are the changes of peripheral blood flow in animals that received vehicle (control, n = 6), received istradefylline (control + istradefylline, n = 4), heatstroke plus vehicle (heatstroke, n = 6), and heatstroke plus istradefylline (heatstroke + istradefylline, n = 7). Data are expressed as mean ± standard error of mean * $P < 0.05$, all versus control

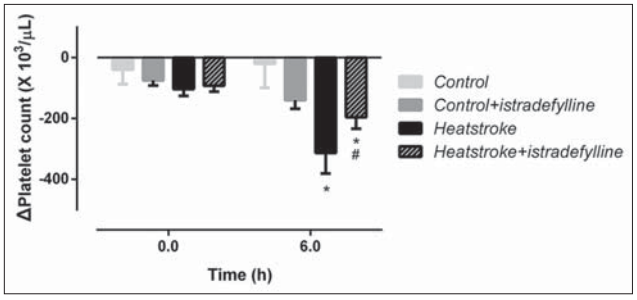

Figure 5: Effects of istradefylline on platelet count in heatstroke rats. Depicted are the changes of platelet number in animals that received vehicle (control, n = 6), received istradefylline (control + istradefylline, n = 4), heatstroke plus vehicle (heatstroke, n = 6), and heatstroke plus istradefylline (heatstroke + istradefylline, n = 7). The platelet count of all groups is normalized with platelet count at 1 h. Data are expressed as mean ± standard error of mean * $P < 0.05$, all versus control; $^{\#}P < 0.05$, with versus without istradefylline in heatstroke rats

of heatstroke rats reached 44.1°C, they were accompanied with significant increase of the peripheral blood flow at time 0 (i.e., immediately after removing them from the heating chamber), mimicking the clinical manifestations of heatstroke patients. Several studies have demonstrated that tachycardia and electrocardiographic changes frequently occur in patients with heatstroke.[20,21] Indeed, the heart rate significantly increased in rats with heatstroke. However, this tachycardia did not significantly change in heatstroke rats treated with istradefylline, indicating that istradefylline could not reverse cardiac abnormalities in heatstroke.

Heatstroke is a medical emergency caused by thermoregulatory failure. Excessive heat accumulation in the body could lead to organ damage and multiple organ dysfunction. Early ICU mortality in heatstroke patients is due to multiple organ failure triggered by systemic inflammatory response.[1,6,7] Indeed, heatstroke rats showed muscle injury, renal dysfunction, and liver dysfunction in this study. The 6-h survival rate of rats with heatstroke was 67%. However, there were no significant improvement in multiple organ dysfunction and survival rate between heatstroke rats and heatstroke rats treated with istradefylline. Thus, istradefylline may not be an effective drug to reduce the mortality associated with multiple organ dysfunction in heatstroke.

The mortality in heatstroke patients is associated with the severity of coagulopathy.[22] Low platelet count is one of the significant factors, which contributes to the mortality of heatstroke patients in the ICU.[4,23] Indeed, the platelet count significantly decreased in rats with heatstroke in this study although the treatment of heatstroke rats with istradefylline significantly ameliorated platelet loss, indicating that istradefylline could have a beneficial effect on platelet in heatstroke. However, this beneficial effect of istradefylline on platelet loss was not enough to counteract the deleterious effects caused by heat stroke on multiple organ dysfunction.

For instance, circulatory failure is also associated with the exacerbation of organ injury in patients with heatstroke.[24,25] Circulatory shock requires the pharmacologic vasopressors to prevent tissue hypoxia and cellular death. Hart *et al*. have reported that the requirement for vasoactive agents is associated with poor outcomes in patients with heatstroke.[26] In addition, Cui *et al*. have observed that heat stress diminishes the blood pressure response in humans.[27] Both findings indicate a close association between low pressor response and poor outcome in heatstroke. Indeed, our results showed that vasoconstrictor responsiveness was significantly attenuated in heatstroke rats. Treatment of heatstroke rats with istradefylline partially, but significantly, improved vascular hyporesponsiveness only at 4 h after heat stress. Thus, although istradefylline partially reversed abnormal vasoconstrictor responsiveness in heatstroke, this short-term improvement was not enough

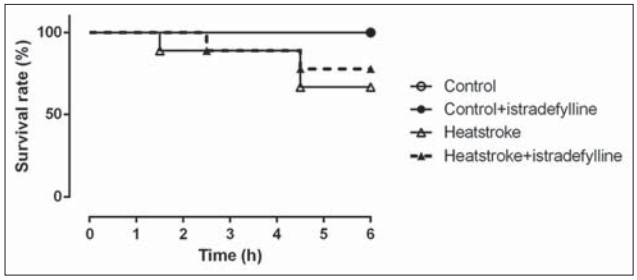

Figure 6: Effects of istradefylline on survival rate in heatstroke rats. Depicted are changes of survival rate in different groups during the experimental period. Control (n = 6); control + istradefylline (n = 4); heatstroke (n = 9); heatstroke + istradefylline (n = 9). Data are expressed as percentage of animals that survived at each time point. $^{*}P < 0.05$, all versus control; $^{\#}P < 0.05$, with versus without istradefylline in heatstroke rats

to reverse the circulatory failure and mortality occurred in heatstroke rats.

To summarize our results, we concluded that istradefylline did not alleviate cardiac abnormalities, hypotension, and multiple organ dysfunction in rats with heatstroke. Even istradefylline had partially increased platelet loss and transiently improved vascular hyporeactivity in heatstroke, both effects were not enough to reverse multiple organ dysfunction and the mortality. Thus, we suggest that the mild impact on abnormal platelet count and pressor response to NE in heatstroke by istradefylline was not able to reduce the heatstroke-induced mortality.

Acknowledgments

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Nil.

Conflicts of interest

There are no conflicts of interest.

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