Empagliflozin Protects Atherosclerosis Progression by Modulating Lipid Profiles and Sympathetic Activity

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Abstract

Background: Several large clinical trials have confirmed the cardioprotective role of Sodium-glucose cotransporter 2 (SGLT2) inhibitors in patients with type 2 diabetes. Here we investigated that empagliflozin, as an SGLT2 inhibitor, could alleviate atherosclerosis progression.

Methods: ApoE-/- mice were fed on a western diet for 12 weeks to induce atherosclerosis. On the 7^{th} week, a group of mice were treated with drinking water containing empagliflozin (10mg/kg/day) while another group was still fed on normal water. On 12^{th} week, the whole aortas of each group were harvested. The Oil red O, HE and movat staining were performed for atherosclerotic lesion area and size. Mouse serum lipid profiles (TC, TG, LDL-C, and HDL-C), systemic inflammation level (IL-1β, IL-6 and IL-10), renin-angiotensin-aldosterone system (RAAS) and sympathetic activity (norepinephrine, and neuropeptide Y) were measured by ELISA.

Results: Empagliflozin reduced the atherosclerotic lesion burden in ApoE-/- mice. Besides, empagliflozin decreased the body weight, lipid profiles, RAAS and sympathetic activity. However, the anti-inflammation effect of empagliflozin was not significantly evident.

Conclusions: Empagliflozin can partly prevent atherosclerosis in ApoE-/- mice, which could be attributed to its inhibition on lipid profiles, and sympathetic activity.

Background

Sodium-glucose cotransporter 2 (SGLT2) is mainly distributed in the proximal tubule of the kidney and responsible for reabsorption of 80%-90% glucose load[1]. SGLT2 inhibitors can reduce glucose reabsorption of proximal tubules and increase urine glucose excretion with high selectivity and specificity, thereby reducing blood glucose levels[2]. With the loss of glucose in the urine, the body weight and blood pressure also decrease significantly [3].

Recent clinical studies have shown that SGLT2 inhibitors can reduce cardiovascular mortality and heart failure hospitalization rates in patients with type 2 diabetes who are at risk of atherosclerotic cardiovascular disease[4–6], which becomes the first hypoglycemic agent to reduce cardiovascular adverse events independent of glycemic control[7]. These data have prompted great interest in the potential mechanisms that mediate the cardioprotective effects of SGLT2 inhibitors. Some studies revealed SGLT2 inhibitor can inhibit inflammation and improve insulin resistance[8] [9], as well as modulate the gut microbiota of type 2 diabetes mice[10]. However, the potential mechanism is still to be explored for the cardioprotective role of SGLT2 inhibitors.

In addition to glycemic control, the mechanism of cardioprotection of SGLT2 inhibitors in patients with type 2 diabetes remains unclear. Ken Lee Chin et al. systematically reviewed preclinical data on the cardioprotective effects of SGLT2 inhibitors and found that reduction of atherosclerosis was one of the underlying mechanisms[11]. Atherosclerosis is a leading cause of adverse cardiovascular events,

including acute coronary syndrome (ACS) and stroke[12]. However, potential role and mechanisms of the SGLT2 inhibitors on atherosclerosis are not fully understood. Therefore, we investigated whether empagliflozin, an SGLT2 inhibitor, can inhibit the development of atherosclerosis and the possible mechanism of its vascular protection.

Materials And Methods

Animals

ApoE-/- mice were obtained from Model Animal Research Center of Nanjing University and housed at the animal room of Nanjing Drum Tower Hospital. To establish an atherosclerosis mouse model, eight-week-old male mice were maintained by a Western diet containing 0.2% (wt/wt) cholesterol and 42% fat (#TP26303, TROPHIC Animal Feed High Tech Co., Ltd, Jiangsu) for 12 weeks. The EMPA group received drinking water containing 10 mg/kg/d of empagliflozin (CAS No. : 864070-44-0, MedChemExpress, China) since the 7th week. The ApoE-/- mice prior to receiving the western diet were set as the control group. All mouse studies were approved by the Nanjing University Animal Care and Use Committee.

Atherosclerotic Lesion Analysis

After 12 weeks, the entire aorta was harvested and observed under a stereomicroscope, and fixed in 4% paraformaldehyde overnight. Then the aorta was opened longitudinally and stained in Oil Red O solution for 2 hours at room temperature. Images were captured using the high-resolution camera. For plaque area analysis in aortic sinus, the upper portion of heart above the line connecting the left and right auricles and proximal aorta was fixed and embedded in paraffin. 10-μm slides were cut and stained with hematoxylin-eosin or Movat (Sevicebio, Wuhan). Images were captured using the Olympus microscope. Lesion size was measured with Images J software (NIH, USA).

ELISA

Mouse serum was collected and stored at -20℃. The lipid profile (TC, TG, LDL, and HDL), inflammatory cytokines (IL-1β, IL-6 and IL-10), RAAS mediators (renin, angiotensin II and aldosterone) and sympathetic mediators (norepinephrine and neuropeptide Y) were measured by ELISA (Jin Yibai Biological Technology Co. Ltd, Nanjing) according to the manufacturer's instructions using standard curve. The optical densities of the samples were detected using a microplate reader (BIOTEK, USA) at a wavelength of 450 nm.

Statistics

Data were shown as mean ± SEM. The 2-taild Student t test was applied for comparison between 2 groups and 1-way analysis of variance with the Bonferroni post hoc test was used for multiple comparisons. $P < 0.05$ was considered statistically significant.

Results

1.Sglt2i attenuates atherosclerotic lesion area

To assess the therapeutic role of SGLT2i in atherosclerosis in mice, apoE-/- mice were fed a Western diet for 12 weeks (AS group), while EMPA group received empagliflozin at a dose of 10 mg/kg/day from 7th to 12th week. Macroscopically, the atherosclerotic lesion size decreased in EMPA group compared with AS group (Fig. 1A). Enface Oil Red O staining also confirmed the presence of reduced atherosclerotic lesion area in EMPA group (Fig. 1B). By HE and Movat staining analysis (Fig. 1C and 1D), empagliflozin significantly reduced lesion size in aortic sinus by ~ 10%.

2. Sglt2i decreases lipid level in atherosclerosis

Excess lipid deposit contributed to the initiation of atherosclerosis. So we evaluated the effect of empagliflozin on lipid profiles. ELISA results showed that empagliflozin could decrease the level of triglyceride (Fig. 2A), total cholesterol (Fig. 2B), and LDL (Fig. 2C). While HDL was not statistically different between groups (Fig. 2D).

3. Sglt2i minimally alleviates systemic inflammation in atherosclerosis

Chronic inflammation is also an important trigger of atherosclerosis. Therefore, we evaluated the systemic inflammation level between groups. Our results found that IL-1β (Fig. 3A) and IL-6 (Fig. 3B) were not significantly decreased in the EMPA group but IL-10 (Fig. 3C). These results suggested that empagliflozin had a low anti-inflammation role.

4. Sglt2i inhibits Renin-Angiotensin-Aldosterone System (RAAS) and sympathetic activity

The chronic activation of Renin-Angiotensin-Aldosterone System (RAAS) was a detrimental factor in the cardiovascular diseases. Our results showed that rennin (Fig. 4A), angiotensin II (Fig. 4B) and aldosterone (Fig. 4C) were increased in AS group while they were inhibited in EMPA group other than angiotensin II. It indicated that empagliflozin could alleviate the activation of RAAS. In addition to RAAS, sympathetic activation also speeds up the progression of atherosclerosis. We found that norepinephrine (Fig. 5A) and neuropeptide Y (Fig. 5B) were partially inhibited in the EMPA group. Interestingly, empagliflozin also decreased the body weight (Fig. 5C) of AS mice to a degree.

Discussion

Our results showed that empagliflozin could mitigate the progression of atherosclerotic plaques in ApoE-/- mice. Body weight and lipid profiles of the empagliflozin-treated group were also lower than those of the untreated group. In addition, we also showed that empagliflozin significantly reduced expressions of norepinephrine (NE) and neuropeptide Y (NPY), as well as renin, angiotensin II, and aldosterone. These results indicate that empagliflozin alleviates the activation of sympathetic activity and RAAS, which

contributes to the development of atherosclerosis. However, the anti-inflammatory effects were not significant in our study.

Previous studies have also demonstrated that SGLT2 inhibitors reduce the development of atherosclerotic lesions in diabetic and non-diabetic mice[13–16]. Our results were consistent with these studies. It was reported that SGLT2 inhibitors could inhibit the activation of NLRP3 inflammasome[17], reduce the secretion of vasoconstrictive eicosanoids and pro-inflammatory chemokines in the vasculature[15, 18], playing an anti-inflammation role. A study showed that empagliflozin prevented the development of atherosclerosis and reduced inflammation and fat deposition in non-diabetic ApoE-/- mice[8]. Our study confirmed that inhibition of sympathetic activity and RAAS contributed to the anti-atherogenic effects of empagliflozin.

Excessive lipid deposition promotes the development of atherosclerosis. However, the effect of SGLT2 inhibitors on lipid profiles is not consistent in animal studies. Several previous studies have shown that SGLT2 inhibitors can lower lipid levels[14, 19, 20], while others have not[21–25]. Our results showed that empagliflozin could reduce the levels of triglyceride, total cholesterol and LDL, while there was no significant difference in HDL between the two groups. Given these findings, further studies are warranted to fully elucidate the effects of SGLT2 inhibitors on lipid metabolism. However, the systemic inflammation level of atherosclerosis in the SGLT2 inhibitor group was not significantly different. Two factors can explain this difference: on the one hand, the non-diabetic ApoE-/- mice we used may not have a significant vascular inflammatory response induced by hyperglycemia. Nakatsu et al demonstrated that hyperglycemia rapidly induced vascular inflammatory response, which can be normalized by short-term (7 days) treatment with the SGLT2 inhibitor luseogliflozin[22]. Even though we did not detect the glucose levels in this experiment. However, previous study have confirmed no significant difference in glucose levels between empagliflozin-treated and untreated mice, and empagliflozin did not increase the risk of hypoglycemia in non-diabetic states [26]. On the other hand, systemic inflammation level is likely affected by the duration of treatment with SGLT2 inhibitors. The duration of our experiment was 12 weeks, and the experimental group was treated with SGLT2 inhibitors since the 7th week. Combining previous studies, we found that SGLT2 inhibitors may inhibit inflammatory mediators with a duration of at least 8 weeks[9, 10, 15, 27]. Therefore, we speculated that short-term of empagliflozin treatment was not enough to play an anti-inflammation effect.

In recent years, some mechanisms underlying the beneficial effect of SGLT2 inhibitor on cardiovascular diseases have been proposed. The decreased toxicity of glucose to endothelial cells may be a potential mechanism in preventing diabetic ApoE-/- mice atherosclerosis[27]. And dapagliflozin could improve the differentiation of epicardial adipose tissue and perivascular adipose tissue[28]. In addition, SGLT2 inhibitor could enhance lipoprotein clearance through heparan sulfate proteoglycans (HSPG) and bile acid pathways[14], which could protect from atherosclerosis progression. In our article, we broaden our understanding of beneficial effect of SGLT2 inhibitor empagliflozin on the progression of atherosclerosis.

Conclusion

In summary, SGLT2 inhibitor empagliflozin may exert a protective role in atherosclerosis by reducing lipid levels and inhibiting sympathetic activity. Due to a relatively short-term treatment, there may be no statistical difference in some experimental results. Future long-term of empagliflozin treatment studies should be performed.

Declarations

Ethic approval and consent to participate

NA

Declarations

Ethic approval and consent to participate

NA

Consent for publication

NA

Availability of data and materials

Yes

Competing interests

All authors declare no conflict of interest.

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None

Authors' contributions

K LN and X B designed this study; L YH and X JM wrote the manuscript; W MY performed the experiments.

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Figures

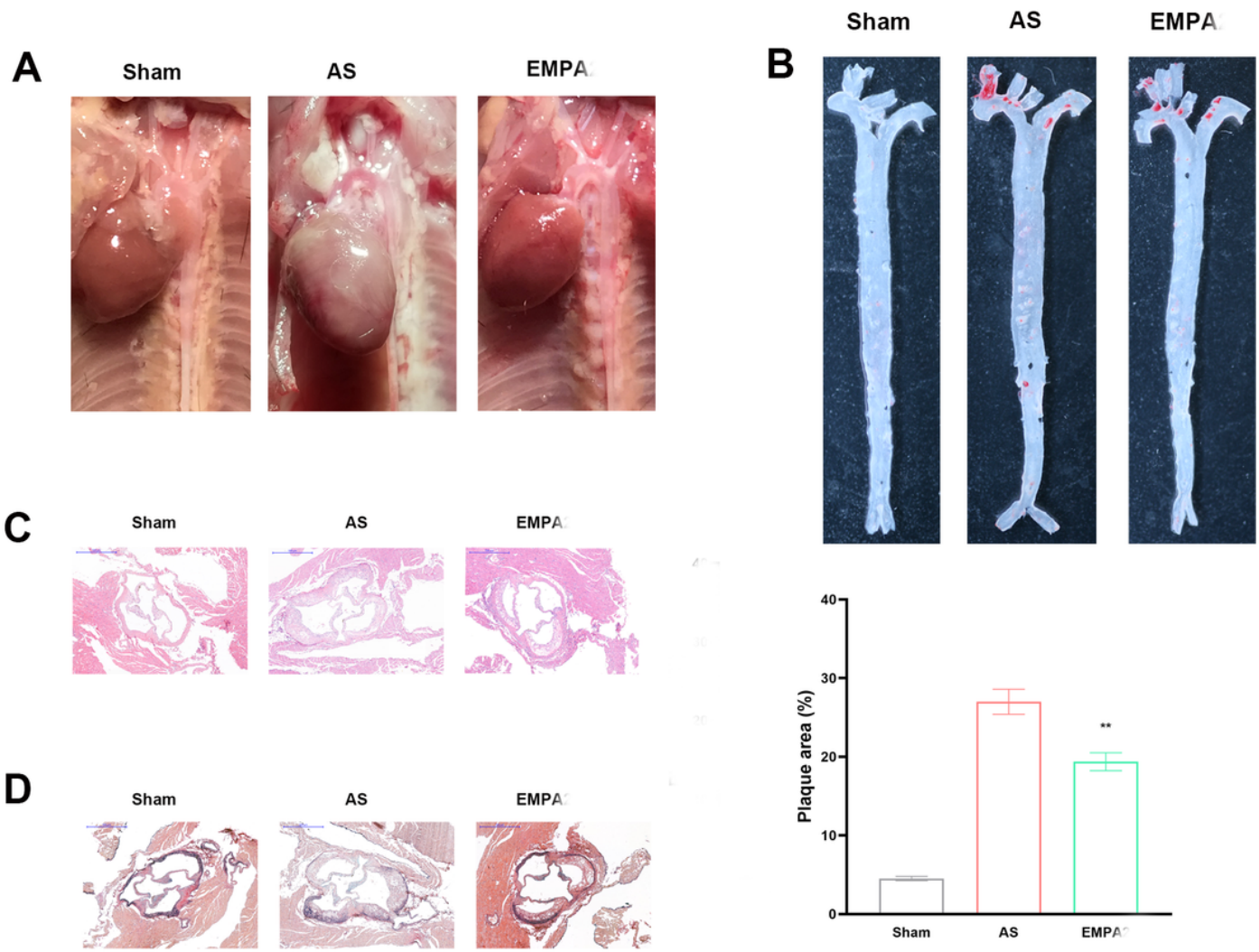

Figure 1

Empagliflozin attenuated atherosclerotic lesion area. Representative microscopical image (A), enface Oil Red O staining (B), HE staining (C), Movat staining (D) of Sham, AS and EMPA group. *p<0.05, **p<0.01, and ***p<0.001.

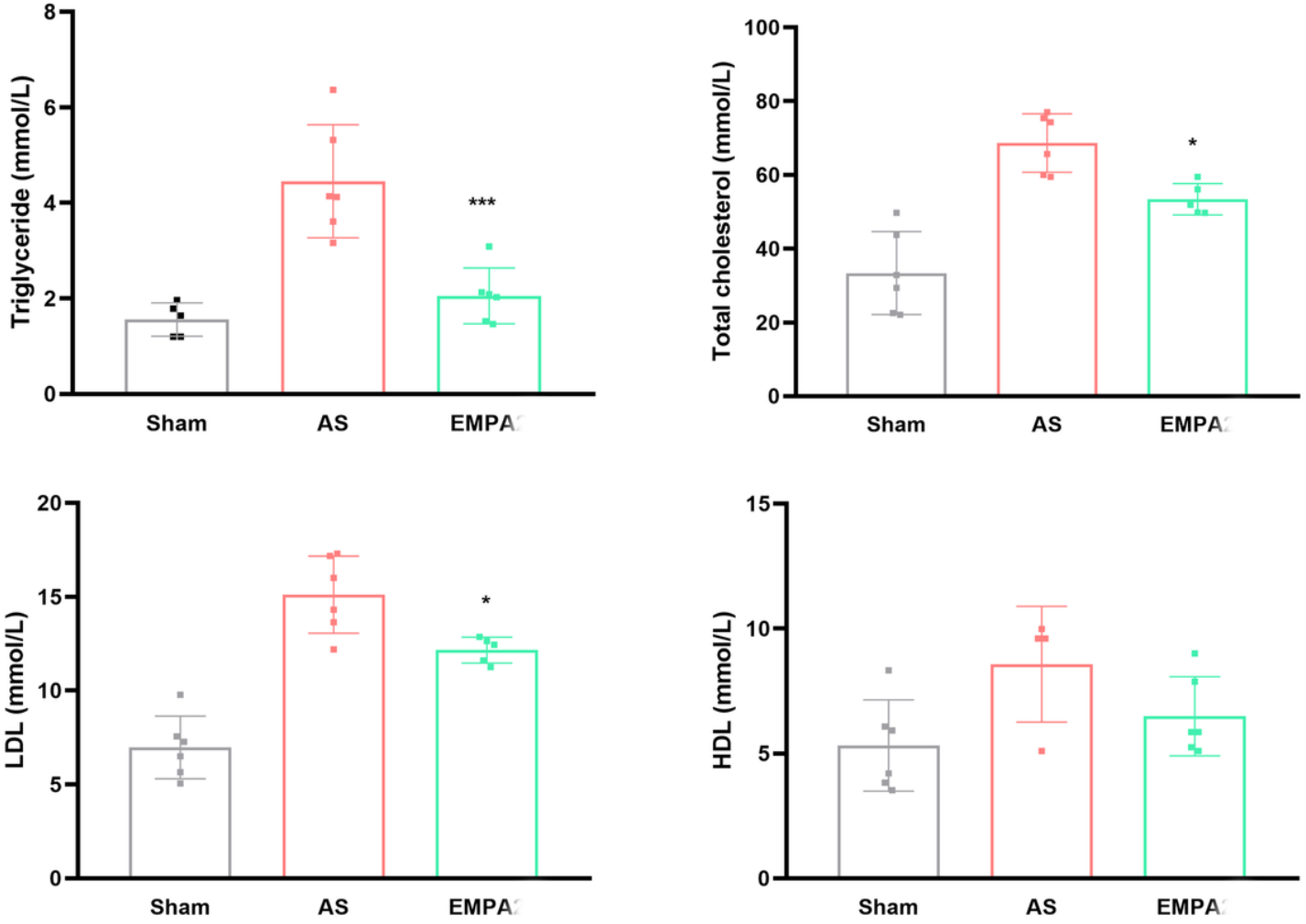

Figure 2

The serum level of triglyceride (A), total cholesterol (B), LDL (C) and HDL (D) between Sham, AS and EMPA group. *p<0.05, **p<0.01, and ***p<0.001.

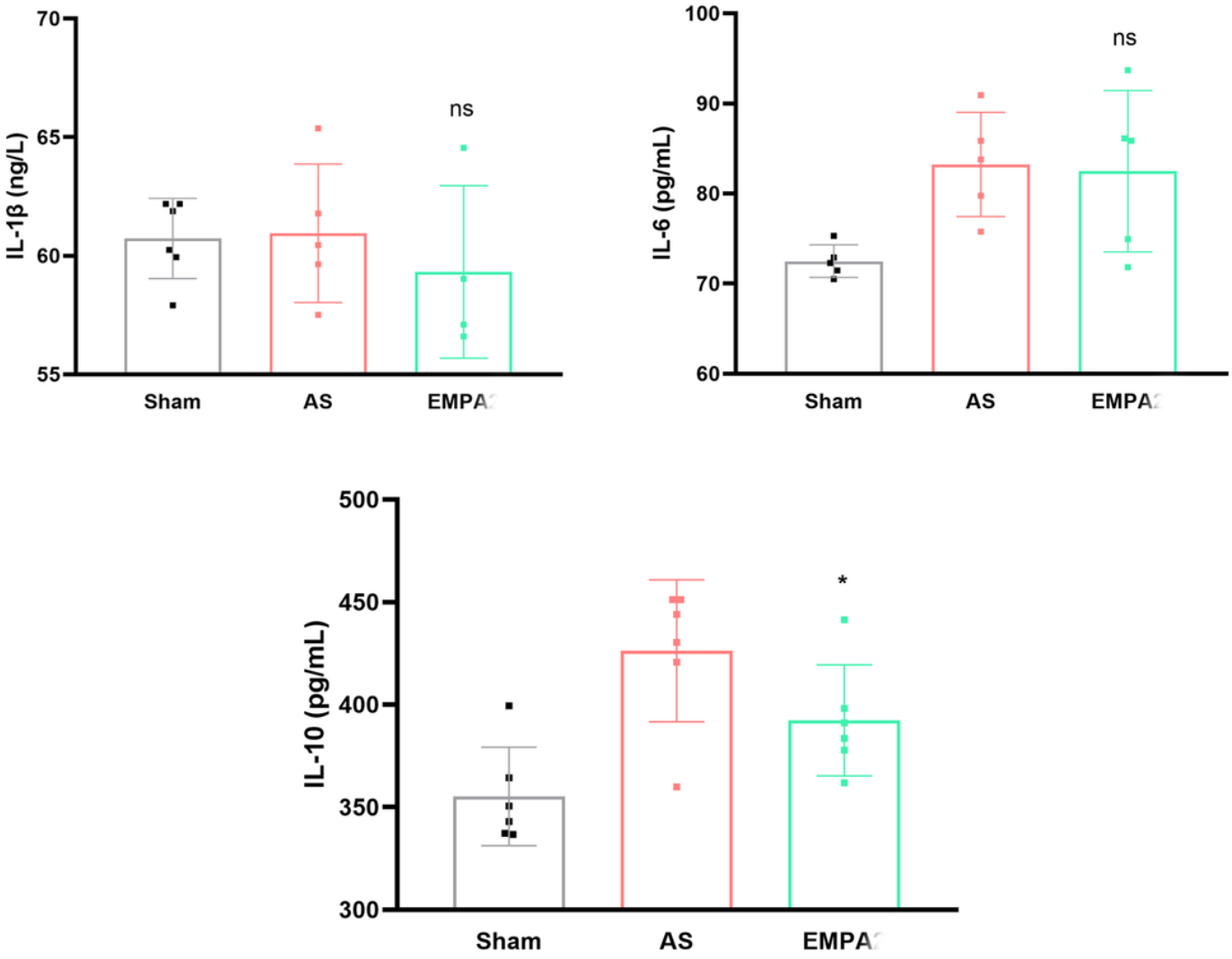

Figure 3

The serum level of IL-1β (A), IL-6 (B), and IL-10 (C) between Sham, AS and EMPA group. *p<0.05, **p<0.01, and ***p<0.001.

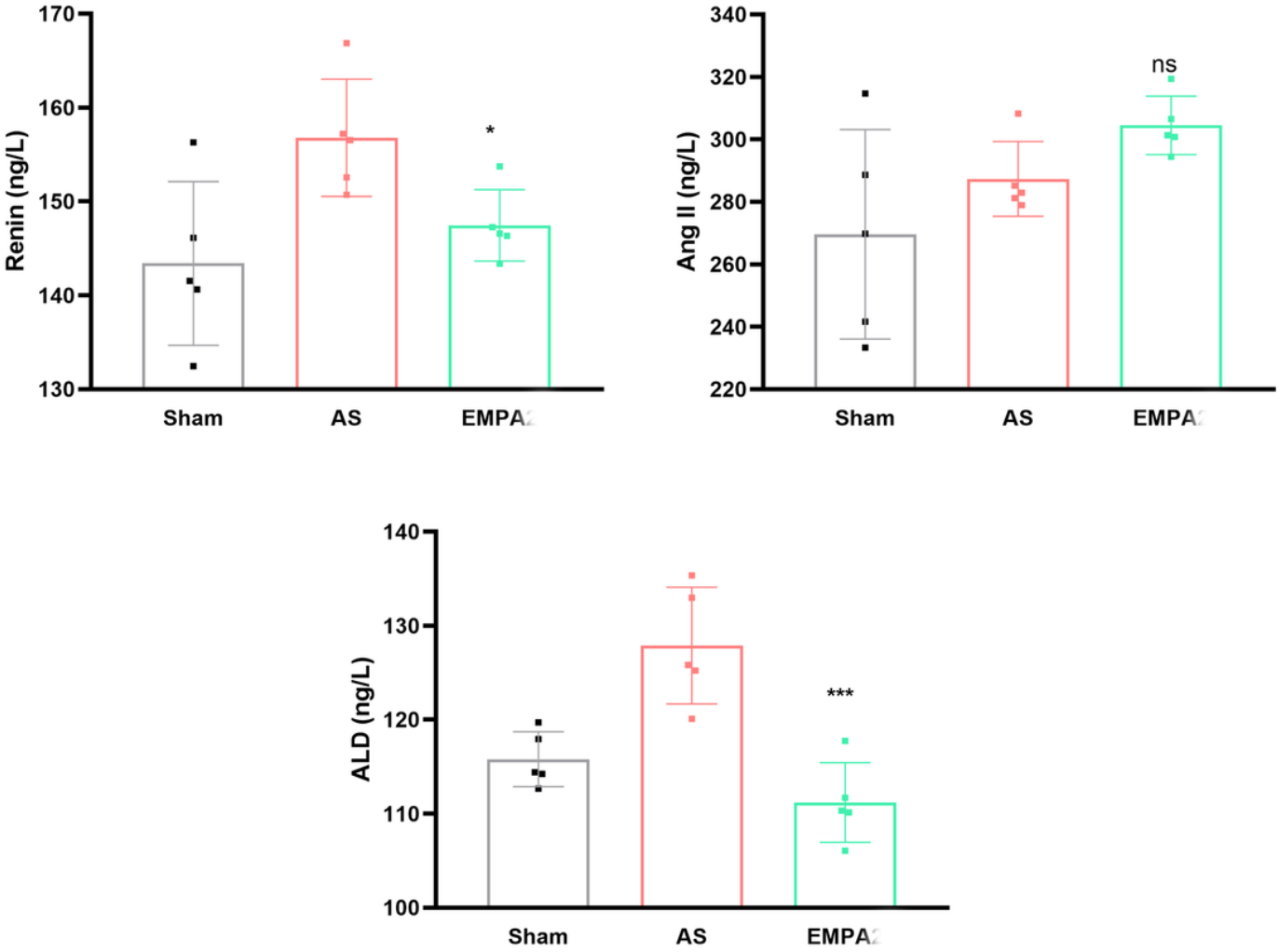

Figure 4

The serum level of rennin (A), angiotensin II (4B) and aldosterone (4C) between groups. *p<0.05, **p<0.01, and ***p<0.001.

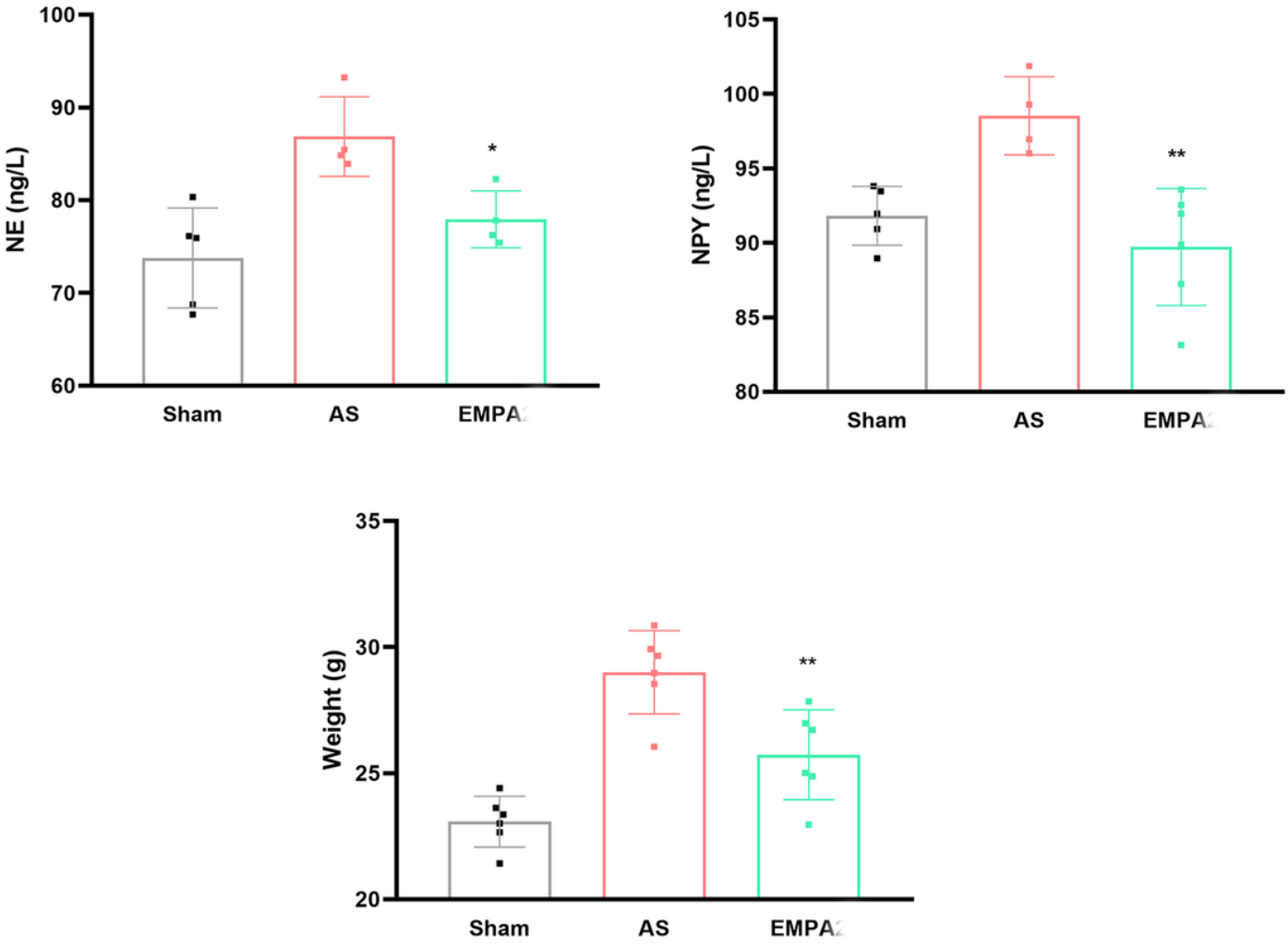

Figure 5

The serum level of norepinephrine (A) and neuropeptide Y (B) and body weight (C) between Sham, AS and EMPA group. *p<0.05, **p<0.01, and ***p<0.001.