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Human tissue Kallikrein 1 rescues erectile function in rats with hyperhomocysteinaemia by protecting endothelial function and inhibiting fibrosis

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Introduction

To investigate the detailed mechanism of erectile dysfunction (ED) induced by hyperhomocysteinaemia (HHcy) in rats and determine whether Human Tissue Kallikrein 1 (hKLK1) might improve it, as we have proved the protective role of hKLK1 on erectile function in aged rats.

Methods

We established a rat model of HHcy through dietary-rich methionine (Met) in male Sprague-Dawley (SD) rats. Male wild-type SD rats (WTR) and transgenic rats harboring the hKLK1 gene (TGR) were fed to 10 weeks of age. Then 24 WTRs were divided into control (n=8), the low-dose (4% Met, n=8), and the high-dose (7% Met, n=8). Another 8 age-matched TGRs with the high-dose formed the TGR+7%Met group. 30 days later, erectile function, level of total homocysteinaemia (tHcy), oxidative stress, endothelial function, cavernous nerve function and fibrosis of all groups were determined.

Table 2 Metabolic parameters

Variable	Control	4%Met	7%Met	TGR+7%Met
Initial weight (g)	307.5±8.9	309.43±11.3	306.15±13.41	310.74±9.53
Final weight (g)	415.41±21.33	366.27±27.34*	342.53±22.82*	350.48±19.98*
Initial tHcy Level (μmol/L)	30.23±4.94	31.72±5.25	30.47±4.33	29.92±4.74
Final tHcy Level (μmol/L)	31.22±5.75	84.49±15.88*	110.77±25.28*	105.84±17.75*
MAP	97.17±11.03	98.73±10.11	100.08±9.37	98.43±8.41

tHcy = total plasma homocysteine; MAP = mean atrial pressure;
Data are shown as mean ± standard deviation; *P <0.05 when compared with the Control group.

Results

1. hKLK1 in the TGR+7%Met group could greatly decrease the tHcy levels and improve ED induced by HHcy in rats.
2. hKLK1 could inhibit oxidative stress of rats HHcy.
3. For the endothelial function, hKLK1 could preserve the endothelial cell-cell junction.
4. hKLK1 could enhance endothelial regeneration and activated the Akt/eNOS signaling pathway.
5. For the fibrosis, hKLK1 could preserve normal corpus cavernosum structure.
6. hKLK1 could preserve normal muscle content through inhibiting apoptosis and promoting autophagy on corpus cavernosum smooth muscle cells.

Conclusion

hKLK1 might effectively improve ED induced by HHcy in rats by protecting endothelial function, promoting cavernous nerve function and inhibiting fibrosis, which suggested hKLK1 might be a potential treatment method for ED.

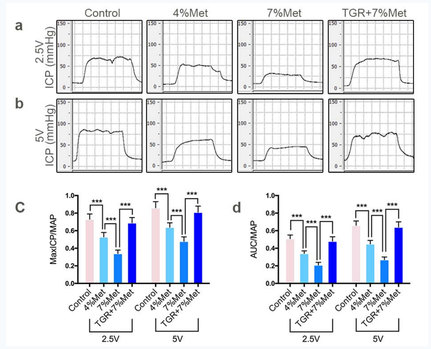

Figure1 Erectile function of rats elicited by cavernous nerve electrical stimulation. (a) and (b): Representative ICP tracing were measured through stimulation of 2.5V and 5 V for 1 minute, respectively. Both ratios of Max ICP and AUC to MAP of all four groups were presented through bar graphs: (c) for Max ICP/MAP, and (d) for AUC/MAP. Data are expressed as mean ± standard deviation. ***P < 0.001.

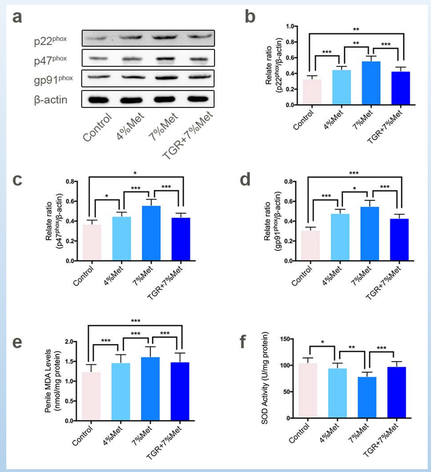

Figure 2 hKLK1 could inhibit oxidative stress in CC of rats with HHcy. (a): Representative Western blot results of $p22^{phox}$, $p47^{phox}$ and $p67^{phox}$ in CC of rats. (b), (c) and (d): Expressions of $p22^{phox}$, $p47^{phox}$ and $p67^{phox}$ with β-actin as the loading control in all four groups were presented through bar graphs. (e) MDA levels determined by the ELISA method in all four groups. (f): SOD activities determined by the ELISA method in all four groups. Data are expressed as mean ± standard deviation. *P < 0.05, **P <0.01 and ***P < 0.001.

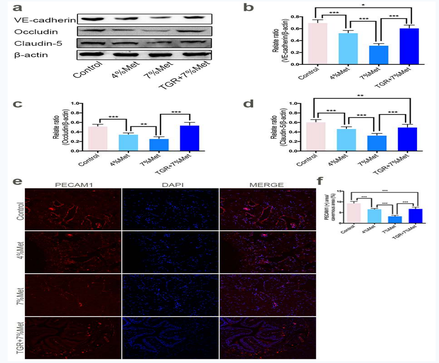

Figure 3 hKLK1 could preserve EC junction protein expressions and endothelial content in CC of rats with HHcy. (a): Representative western blot results for VE-cadherin, Occludin and Claudin-5 in CC of rats of all four groups. (b), (c) and (d): Expressions of VE-cadherin, Occludin and Claudin-5 with β-actin as the loading control in all four groups were presented through bar graphs. (e): Immunofluorescence results of PECAM-1 in rats of all four groups. (f): Ratios of PECAM-1 positive area to cavernous area were presented through bar graphs.

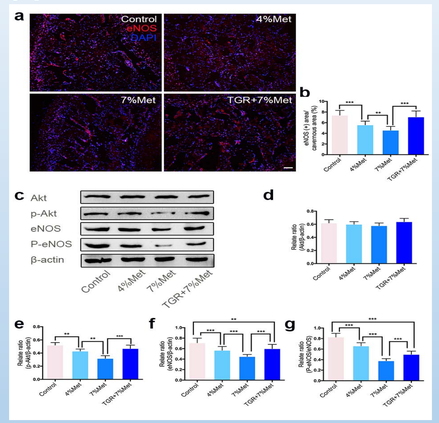

Figure 4 hKLK1 activates Akt/eNOS signaling pathway in CC of rats with HHcy. (a): Immunofluorescence results of eNOS in CC of rats of all four groups. (b): Ratios of eNOS positive area to cavernous area were presented through bar graphs. (c): Representative Western blot results for Akt, p-Akt, eNOS and p-eNOS in rats of all four groups. (d), (e), (f) and (g): Expressions of Akt, p-Akt, eNOS and p-eNOS with β-actin as the loading control in all four groups were presented through bar graphs.

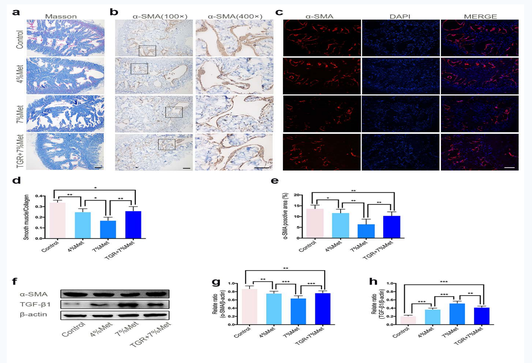

Figure 5 hKLK1 could reduce fibrosis in CC of rats with HHcy. (a): Masson's trichrome staining results of CC of rats in all four groups. (b): Immunohistochemical staining result of α-SMA in CC of rats (magnification ×100 and ×400). (c): Immunofluorescence results of α-SMA in CC of rats. (d): Ratios of Smooth muscle to Collagen were presented through bar graphs. (e): Ratios of α-SMA positive area to cavernous area were presented through bar graphs. (f): Representative Western blot results for α-SMA and TGF-β1 in CC of rats. (d) and (e): Expressions of α-SMA and TGF-β1 with β-actin as the loading control in all four groups were presented through bar graphs.

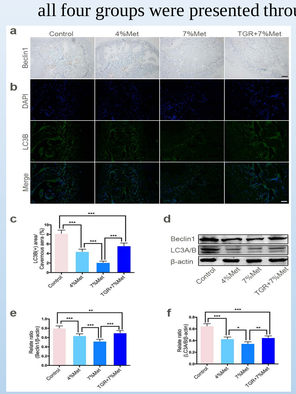

Figure 6 hKLK1 could promote autophagy in CC of rats with HHcy. (a): Immunofluorescence results of Beclin1 in CC of rats. (b): Immunofluorescence results of LC3B in CC of rats. (c): Ratios of LC3B positive area to cavernous area were presented through bar graphs. (d): Representative Western blot results for Beclin1 and LC3A/B in CC of rats. (e) and (f): Expressions of Beclin1 and LC3A/B with β-actin as the loading control in all four groups were presented through bar graphs.

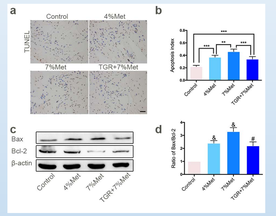

Figure 7 hKLK1 could reduce apoptosis in CC of rats with HHcy. (a): Apoptosis levels in all four groups was determined by TUNEL method. (b): Apoptosis index was presented through bar graph. (c): Representative Western blot results for Bax and Bcl-2 in CC of rats. (d): Ratios of Bax to Bcl-2 with β-actin as the loading control in all four groups were presented through bar graphs.