Added Benefit of Empagliflozin: Improvement of Erectile Dysfunction in Diabetic Type II Rats

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OBJECTIVES

- Chronic hyperglycemia correlates with the occurrence and severity of urological functional complications such as erectile dysfunction (ED) [1].
- ED significantly impacts the quality of life of diabetic patients who are poor responders to PDE5 inhibitors [2].
- It is hypothesized that empagliflozin, an SGLT-2 inhibitor, could ameliorate type 2 diabetes-associated ED through effective glycemic control.

Aims of the study:

Assess the effects of chronic empagliflozin:

- *In vivo* on erectile function in the Goto-Kakizaki rat (GK), a validated model for type 2 diabetes-associated ED [3].
- *Ex vivo* on endothelium-dependent, -independent and nitrergic relaxations of cavernosal strips from GK rats.

MATERIALS & METHODS

Experimental design

- At 15 weeks of age, Wistar rats and a subgroup of GK rats were fed with a control diet, and additional GK rats were fed with a control diet premixed with empagliflozin (25.3 ± 0.9 mg/kg/day) for 28 days.
- On day 26 of treatment, subgroups of rats were placed in metabolic cages for 48 hours. During the last 24 hours, urine was collected to determine 24-hour diuresis, glycosuria and creatinine clearance.
- On day 29 of treatment and 16 hours after fasting, rats underwent *in vivo* evaluation of their erectile function under anesthesia. Thereafter, blood samples were collected to assess HbA1c and plasma inflammation biomarkers (Multiplex assay). After euthanasia, the corpus cavernosum of diabetic GK rats and control Wistar rats were immediately harvested for *ex vivo* isometric tension studies.

***In vivo* evaluation of erectile function via electrical stimulation of the cavernous nerve (ES CN) [4]**

- After 5 minutes of baseline recording of simultaneous computerized measurement of mean arterial pressure (MAP) and intracavernous pressure (ICP), the cavernous nerve (CN) was stimulated (6 V, 1 ms for 45 s) at different frequencies (0, 2.5, 5, 7.5, 10, 12.5 and 15 Hz) at 3-minute intervals in a randomized manner in order to assess the erectile responses.
- Erectile responses to ES CN were expressed as a ratio of ICP (mmHg) / MAP (mmHg) x 100, ICP being the difference between ICP in the flaccid state, i.e. before stimulation and ICP during the plateau phase of the erectile response, and MAP, the mean arterial pressure during the plateau phase, and as the ratio of AUCtot / MAP, AUCtot being the area under the curve during the whole erectile response.

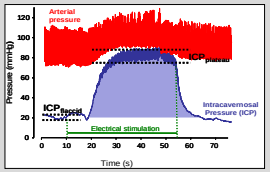


Illustration of the parameters computed for *in vivo* erectile function evaluation following ES CN [4].

***Ex vivo* experiments on isolated strips of corpus cavernosum**

Cavernosal strips were obtained and placed in organ chambers for isometric tension studies.

- **Endothelium-dependent relaxations:** Concentration-response curves (CRC) for acetylcholine (ACh) were performed on phenylephrine precontracted cavernosal strips by cumulative addition of increasing drug concentrations (ACh 10-9 to 10-4 M) to the baths in semi-log increments.
- **Nitrergic relaxation responses to electrical-field stimulation (EFS):** Frequency-response curves (FRC) to EFS were performed on phenylephrine precontracted cavernosal strips by successive stimulation of the strips at different electrical parameters (1 ms - 10 s - 300 mA, 1, 2, 4, 8, 16 and 32 Hz).
- **Endothelium-independent relaxations:** CRCs for sodium nitroprusside (SNP) were performed on phenylephrine precontracted cavernosal strips by cumulative addition of increasing drug concentrations (SNP 10-9 to 10-5 M) to the baths in log increments.

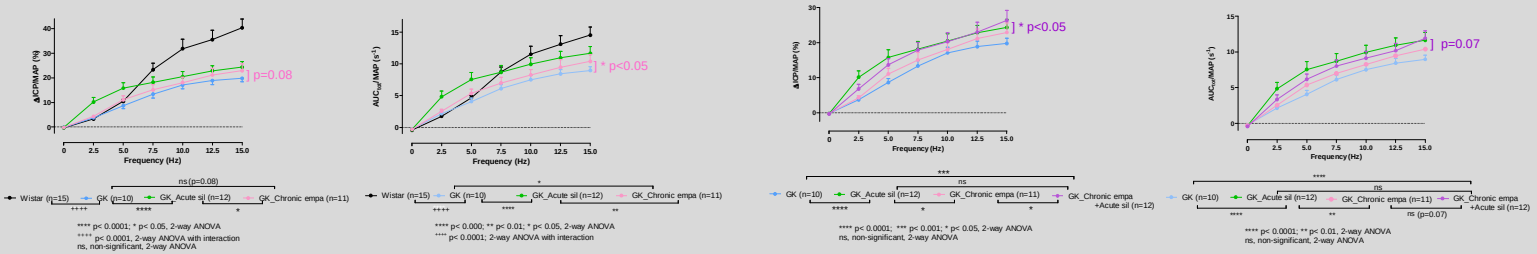
Statistical analysis

- All results are presented as mean ± SEM. For erectile function evaluation, comparisons of frequency-response curves were performed with a two-way ANOVA statistical analysis test. For *ex vivo* experiments, statistical comparisons of the CRCs or FRCs were performed using a two-way ANOVA statistical analysis test. Statistical analysis was performed with GraphPad Prism® 5.04 software. P values <0.05 were considered significant.

RESULTS

Effect of empagliflozin on *in vivo* erectile function in GK rats

Erectile responses elicited by ES CN at increasing stimulation frequencies in anaesthetized Wistar and GK rats



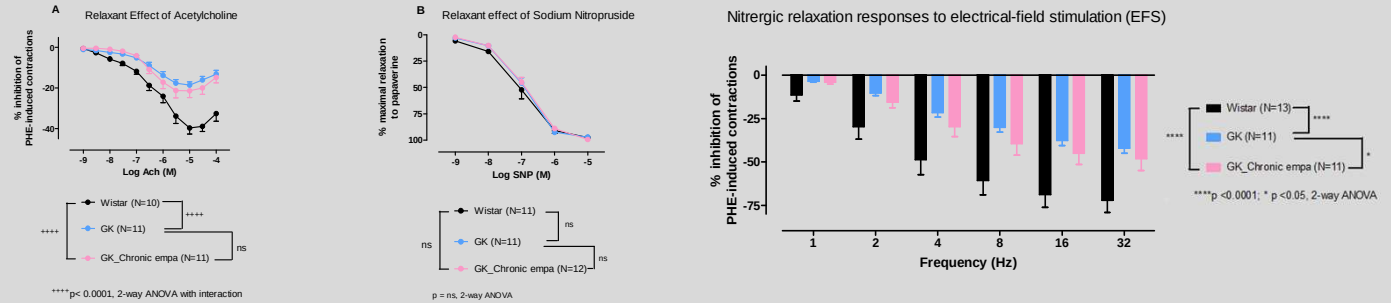
➡ The erectile responses elicited by ESCN were considerably decreased in GK compared to Wistar rats

➡ Chronic empagliflozin at 25 mg/kg/day significantly improved erectile responses of GK rats

➡ Acute dosing of sildenafil significantly improved erectile function of GK rats

➡ Acute dosing of sildenafil further potentiated the beneficial erectile effect of empagliflozin treatment in GK rats

Effect of empagliflozin on endothelium-dependent, -independent and nitrergic relaxations of cavernosal strips of GK rats



➡ Empagliflozin did not elicit discernable changes on endothelium-dependent and -independent relaxations in isolated cavernosal strips of diabetic GK rats

➡ Empagliflozin improved the nitrergic relaxations of cavernosal strips from diabetic GK rats

Metabolic parameters and inflammatory biomarkers

	Wistar	GK	GK_Chronic empa
Inflammatory biomarkers			
CRP (µg/ml)	4467 ± 378.6 (n=12)	5189 ± 298.8 (n=12)	4867 ± 761 (n=12)
TNFα (pg/ml)	10.7 ± 1.5 (n=13)	9.5 ± 2.6 (n=8)	2.3 ± 0.6 && (n=8)
MCP-1(pg/ml)	3195 ± 245.1 (n=14)	4760 ± 284* (n=12)	4027 ± 279 ns (n=12)

*Data are mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, versus age-matched Wistar rats, Student's t-test. &p<0.05, &&p<0.01, &&&p<0.001 versus GK rats, Student's t-test.*

	Wistar	GK	GK_Chronic empa
Metabolic parameters			
HbA1c (%)	5.8±0.1 (n=14)	9.0±0.5***(n=11)	6.8±0.1&&& (n=12)
Glycosuria (g/24h)	4.0±0.2 (n=15)	865.6±314.2**(n=12)	6752.0±370.6&&& (n=11)
Creatinine clearance (ml/min)	0.6±0.0 (n=14)	0.5±0.0** (n=12)	0.6±0.0&& (n=10)
Diuresis (ml/24h)	14.4±1.1 (n=15)	24.1±4.9* (n=12)	64.2±4.1&&& (n=11)

*Data are mean ± SEM. *p<0.05 versus age-matched Wistar rats, Student' s t-test. &&p<0.01, nsp=0.07 versus GK rats, Student's t-test.*

➡ Empagliflozin increased urinary glucose excretion in diabetic GK rats and consequently decreased the percentage of HbA1c

➡ Empagliflozin restored creatinine clearance in diabetic GK rats to the level of control Wistar rats suggesting a renoprotective effect in type 2 diabetes

➡ Plasma levels of inflammatory biomarkers (CRP and MCP-1) tended to be decreased with empagliflozin treatment

➡ Empagliflozin significantly decreased plasmatic levels of TNFα

CONCLUSIONS

1. As expected, 4 weeks treatment with empagliflozin (25.3 ± 0.9 mg/kg/day) improved diabetic status (i.e. HbA1C) of diabetic type II GK rats and decreased diabetes-associated inflammatory state via increased urinary glucose excretion. Moreover, empagliflozin restored creatinine clearance in diabetic GK rats to a level comparable to that measured in control Wistar rats suggesting that it has a renoprotective effect.

2. In this validated preclinical model of erectile dysfunction in diabetic type II GK rats:
   - Empagliflozin improved *in vivo* erectile responses to electrical stimulation of the cavernous nerve.
   - This beneficial effect of empagliflozin to improve erectile function is associated with an improved nitrergic relaxation of cavernosal strips.
   - Moreover acute dosing of sildenafil further increased the erectile response of rats treated with empagliflozin.

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