Abstract # AM18-3448 . Low intensity-Shockwave Therapy (Li-ESWT) delivered by Aries® improves erectile function and decreases cavernosal fibrosis of spontaneously hypertensive rats (SHR)

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PELVI PHARM

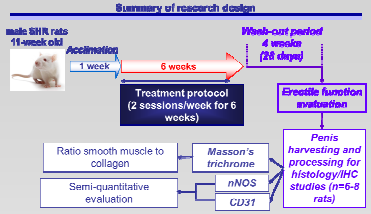
OBJECTIVES

- Hypertension is a risk factor for erectile dysfunction in men.
- Low intensity extracorporeal shock waves therapy (Li-ESWT) has been reported to improve erectile function in patients with moderate or severe ED or even convert phosphodiesterase type 5 inhibitors (PDE5is) non-responders to responders
- Efficacy of Li-ESWT has been reported in models of ED in diabetic rats [1], but studies of Li-ESWT on hypertension associated ED models have not been conducted so far.

Aim of the study:

- To investigate whether Li-ESWT could further enhance in vivo erectile responses following acute sildenafil administration in the spontaneously hypertensive rats (SHR), a validated model for hypertension-associated ED [2-3]
- To explore the mechanism(s) of action of Li-ESWT by histological and immuno-histological evaluation of cavernosal smooth muscle/conjunctive tissue ratio (Masson's trichrome), penile microcirculation (CD31) and non-adrenergic non-cholinergic neurons (nNOS).

MATERIALS & METHODS



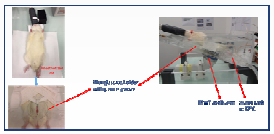
Group #	Groups	Rat strain	Li-ESWT treatment	Acute treatment during erectile function testing	N=
I	SHR	SHR	Sham	Sildenafil 0.3mg/kg	14
II	SHR+ESWT		ESWT	Sildenafil 0.3mg/kg	14

- Male SHR (n=12/group) were used at 11 weeks of age.
- ED function and ex-vivo experiments

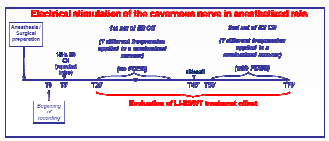
All procedures are performed in compliance with the legislation on the use of laboratory animals (NIH publication N°85-23, revised 1996) and Animal Care Regulations in force in France as of 1988 (authorization from competent French Ministry of Agriculture - Agreement No. B78-423-1, July 2017).

Li-ESWT Treatment protocol
Male SHR rats received 2 sessions of Li-ESWT per week for 6 weeks. Shockwaves were delivered by a calibrated probe yielding a controlled energy flux density of 0.06mJ/mm2 attached to a compact electrohydraulic unit with a focused shockwave source (Aries®, Dornier Medtech, Germany).
To facilitate coverage and transmission of the shockwaves, the penis of each anesthetized rat was manually stretched and dipped into a specifically designed water-filled tank. Following a 4-week wash-out period, erectile function was assessed by electrical stimulation of the cavernous nerve in rats under anesthesia.

Erectile function evaluation: electrical stimulation of the cavernous nerve (ES CN) [4]
After 5 minutes of baseline recording of simultaneous computerized measure of mean arterial pressure (MAP) and intracavernous pressure (ICP), the CN was stimulated (6 V, 1 ms for 45 s) at different frequencies (0, 2, 3, 4, 5, 7.5 and 10Hz) 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 and AUC45 / MAP, AUCtot and AUC45 being the area under the curve during the whole erectile response or the first 45 s during ES CN

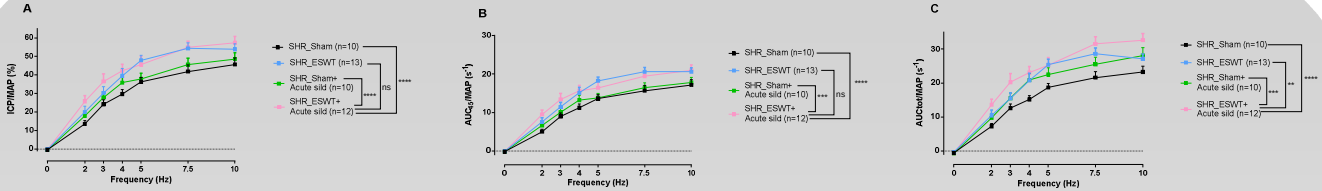


Li-ESWT treatments delivered using a special probe attached to a compact electrohydraulic unit with a focused shockwave source to the penis of anesthetized rat dipped into a water-filled tank



Experimental design of ES CN investigation

Effect of Li-ESWT on erectile responses elicited by ES CN in SHR



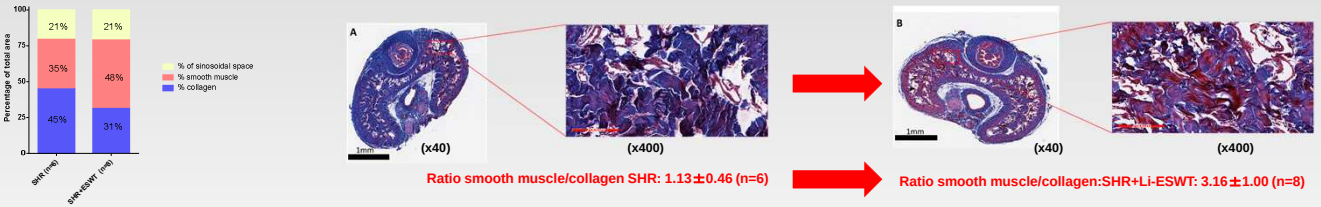
➡ The erectile responses were significantly increased in SHR treated with acute sildenafil at 0.3mg/kg

➡ Li-ESWT delivered by Aries® significantly increased erectile responses of SHRs .

➡ Li-ESWT delivered by Aries® significantly potentiated the pro-erectile effect of acute sildenafil in SHRs.

➡ **Interestingly, the combination of ESWT and acute sildenafil further improved significantly the erectile responses of SHRs of either therapy alone in terms of AUCtot/MAP :**
At 10Hz: +20% compared to Li-ESWT therapy alone and +16% compared to acute sildenafil alone

Effect of Li-ESWT on smooth muscle/conjunctive tissue ratio by Masson's trichrome histological analysis



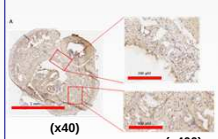
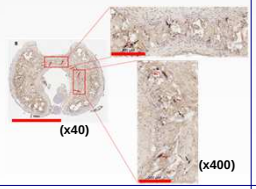
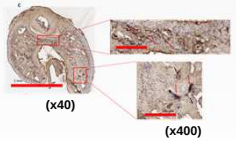
Ratio smooth muscle/collagen SHR: 1.13±0.46 (n=6)

Ratio smooth muscle/collagen:SHR+Li-ESWT: 3.16±1.00 (n=8)

➡ Li-ESWT induces morphological remodelling of the corpus cavernosum with an increase in smooth muscle content and a decrease in collagen content

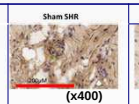
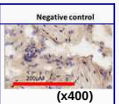
➡ Li-ESWT led to a 2.5 fold increase in the ratio of smooth muscle to collagen

Immunolabeling of CD31 in cavernosal tissue sections

	Negative control	Sham SHR	SHR+ESWT
Representative penile section	 (x40) (x400)	 (x40) (x400)	 (x40) (x400)
Semi-quantitative analysis (mean number of dots per section counted by a blinded experimenter)		29.8±2.2	34.8±1.6

➡ SHRs which received two sessions / week for 6 weeks of ESWT displayed increased CD31 expression in corpus cavernosum compared to sham SHRs while nNOS-positive nerves remained unchanged.

Immunolabeling of nNOS in cavernosal tissue sections

	Sham SHR	SHR+ESWT	Negative control
Representative penile section	 (x400)	 (x400)	 (x400)
Semi-quantitative analysis (mean score attributed by a blinded experimenter)	1.5±0.2	1.5±0.1	

Arrows point at representative CD31 or nNOS_positive stained in brown.

CONCLUSIONS

- ✓ This study shows that acute sildenafil significantly improved erectile responses elicited by ES CN in SHR, in line with clinical findings reporting a response to PDE5is in hypertension-associated ED.
- ✓ To our knowledge, this study is the first to clearly shows the pro-erectile facilitator effect of ESWT in a model of hypertension-associated ED, the SHR, and thereby confirms the therapeutic benefit of ESWT in ED of vascular origin
- ✓ Interestingly, this study reports that Li-ESWT delivered by Aries® was able to potentiate the pro-erectile effect of acute sildenafil in hypertension-associated ED, indicating that combining PDE5i with Li-ESWT delivered by Aries® could salvage PDE5is non responders.
- ✓ This pro-erectile effect was accompanied by (1) an increase in cavernosal smooth muscle and a decrease in collagen contents leading to an increase in the smooth muscle/collagen ratio and (2) an increase in vascular density.

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1. **Assaly-Kaddoum R, Giuliano F, Laurin M, Gorny D, Kergoat M, Bernabe J, Vardi Y, Alexandre L, Behr-Roussel D.** Low Intensity Extracorporeal Shock Wave Therapy Improves Erectile Function in a Model of Type II Diabetes Independently of NO/cGMP Pathway. J Urol 2016; 196:950-956
2. **Oudot A, Oger S, Behr-Roussel D, Caisey S, Bernabe J, Alexandre L, Giuliano F.** A new experimental rat model of erectile dysfunction and lower urinary tract symptoms associated with benign prostatic hyperplasia: the testosterone-supplemented spontaneously hypertensive rat. BJU Int 2012; 110:1352-1358.
3. **Behr-Roussel D, Gorny D, Mevel K, Compagnie S, Kern P, Sivan V, Bernabe J, Bedigian MP, Alexandre L, Giuliano F.** Erectile dysfunction: an early marker for hypertension? A longitudinal study in spontaneously hypertensive rats. Am J Physiol Regul Integr Comp Physiol 2005; 288:R276-R283.