Targeting both the dynamic and static components of LUTS/BPH using a soluble guanylate cyclase stimulator compared to vardenafil: preclinical evidences

D.Behr-Roussel[1,2], A. Oudot[1,2], S. Oger-Roussel[1,2], D. Gorny[1,2], P. Sandner[3], J. Bernabé[1,2], L. Alexandre[1], F. Giuliano[2,4]

[1] Pelvipharm, Orsay, France; [2] EA 4501 Université Versailles Saint Quentin en Yvelines, Garches, France; [3] Bayer Pharma AG, Wuppertal, Germany; [4] AP-HP, Neuro-Uro-Andrology, Dept. of Physical Medicine and Rehabilitation Raymond Poincaré Hospital, Garches, France

BACKGROUND

In randomized clinical trials, PDE5 inhibitors improve LUTS related to BPH.

Soluble guanylate-cyclase (sGC) stimulators bypass the need for an NO drive while activating the same signaling pathway. We have assessed and compared BAY 41-2272, a sGC stimulator, to vardenafil with respect to their relaxing effects on human prostate and their antiproliferative / pro-apoptotic effects on prostate growth induced by testosterone supplementation in the rat.

OBJECTIVES

- To assess and compare BAY 41-2272, a sGC stimulator, to vardenafil with respect to their relaxing effects on human prostate
- To evaluate quantitatively the chronic effects of the sGC stimulator, BAY 41-2272, and vardenafil on the prostatic expression of proliferation and apoptosis markers in the testosterone-induced rat model of BPH

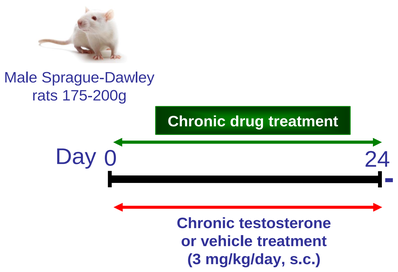
MATERIALS & METHODS

Human prostate samples were obtained from 12 patients (67±2.8 years old) undergoing cystoprostatectomy for infiltrating bladder cancer. Prostatic strips were mounted isometrically in organ baths filled with Krebs-HEPES buffer containing indomethacin (10^{-5} M) and dexamethasone (10^{-5} M) maintained at 37°C and bubbled with 95% O2 and 5% CO2, pH 7.4.

Firstly, cumulative concentration-response curves were performed in order to evaluate and compare the effects of BAY 41-2272 and vardenafil on PHE (10^{-5} M)-precontracted or KCl (50 mM)-precontracted human prostatic strips.

Secondly, the potential enhancing effect of BAY 41-2272 on SNP-induced relaxations of PHE-precontracted prostatic strips was determined after exposure of the strips to BAY 41-2272 (10^{-6} or 10^{-5} M) for 20 min.

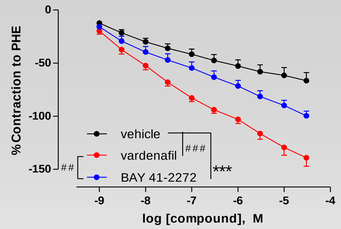
Relaxation responses are expressed as a percentage of inhibition of the contractile response to PHE or KCl. For each CRC, a pD2 value (-log concentration of compound that produces 50 % reduction of the maximal response) and a mean maximal effect (E_{max}) are determined using the four parameters logistic regression using GraphPad Prism® 5.04 software.

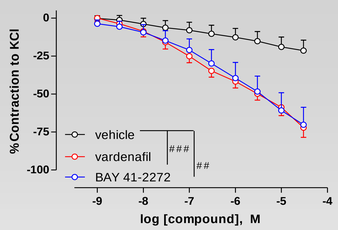


Adult male Sprague-Dawley rats (n=40) were treated for 24 days by daily subcutaneous injection with testosterone (3 mg/kg) or sesame oil together with an oral gavage twice a day of BAY 41-2272 (0.3mg/kg), vardenafil (3mg/kg), or vehicle (10 % Transcutol / 20 % Cremophor / 80 % tap water). At the end of the treatment period, lateral lobes of the prostates were harvested, homogenized and evaluated for the expression of cleaved caspase-3 and -9, markers of apoptosis and PCNA and cyclin D1, markers of proliferation by western blot on a denaturing SDS-polyacrylamide gel. After electroblotting onto PVDF membrane (Millipore, France) (Trans-blot® SD Semi-Dry Transfer Cell Bio-Rad) and immunodetection, proteins of interest were detected by enhanced chemiluminescence and exposed to autoradiographic films. The films were then scanned and relative density against an internal control determined (NIS-Elements, Nikon, France) to allow inter-membrane comparisons. Results are expressed as mean ± SEM. Comparisons were performed with a Student's t-test and P values < 0.05 were considered significant.

RESULTS

Ex vivo experiments on human prostate samples



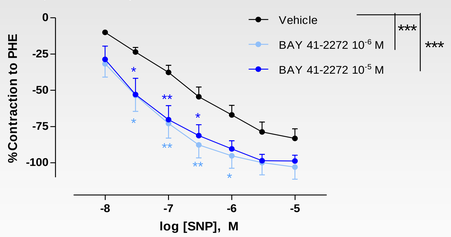


Comparison of effect of BAY 41-2272 and vardenafil on PHE- or KCl-induced contractions of human prostatic strips. *p = ns, ### p < 0.001, two-way ANOVA with interaction and *** p < 0.001 two-way ANOVA .*

Phenylephrine-induced precontractions				
	pD2 **	*E_{max} ns (%)*	*PHE ns (mg)*	*N*
CRC to vehicle	_	_	479 ± 204	8
CRC to vardenafil	7.6 ± 0.1 †	> 100 † (#)	262 ± 83	6
CRC to BAY 41-2272	6.9 ± 0.2	-90.0 ± 4.3	317 ± 38	6

KCl-induced precontractions				
	pD2 **	*E_{max} ns(%)*	*KCl ns (mg)*	*N*
CRC to vehicle	_	_	498 ± 84	11
CRC to vardenafil	6.6 ± 0.2 §	-62.9 ± 6.0	498 ± 60	6
CRC to BAY 41-2272	6.1 ± 0.2 §§	-70.3 ± 10.7	555 ± 211	8

Potency (pD2) and maximum response (E_{max}) obtained using four-parameters logistic regression for vardenafil and BAY 21-4272-induced relaxations on PHE or KCl-induced contractions of human prostatic strips. *Data are mean ± SEM of N experiments using prostate samples from N different patients. p = ns, ** p < 0.01, one-way ANOVA followed by Newman-Keuls post-hoc test, † p < 0.05, ††† p < 0.001 versus BAY 41-2272.*



Effect of BAY 41-2272 on SNP-induced relaxation on PHE-elicited prostatic contractions. *** *p < 0. 001, two-way ANOVA followed by Bonferroni post tests: * p < 0.05; ** p < 0.01.*

➡ **Vardenafil and BAY 41-2272 induce significant concentration-dependent relaxations of both PHE- or KCl-precontracted human prostatic strips with a greater effect of vardenafil on PHE-precontracted prostatic strips. BAY 41-2272 enhances the relaxant effect of SNP on PHE pre-contracted human prostatic strips.**

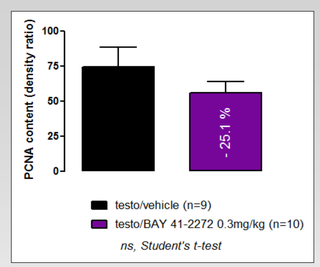
In vivo experiments in testosterone-supplemented rats

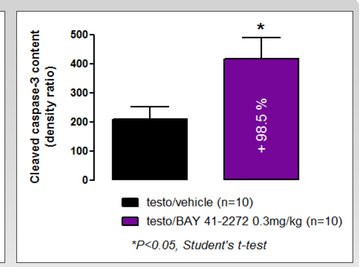
Total prostate weight (mg/100 g BW)		*N*
Sesame oil/veh	203 ± 6	10
Testo/veh	302 ± 11	10
Testo/vardenafil	308 ± 13	10
Testo/BAY 41-2272	329 ± 10	10

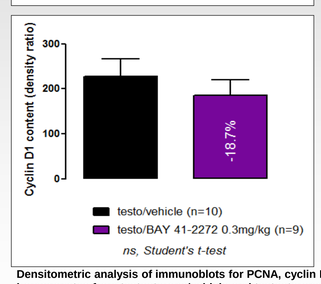
Lateral lobes weight (mg/100 g BW)		*N*
Sesame oil/veh	133 ± 6	10
Testo/veh	189 ± 9	10
Testo/vardenafil	196 ±10	10
Testo/BAY 41-2272	207 ± 7	10

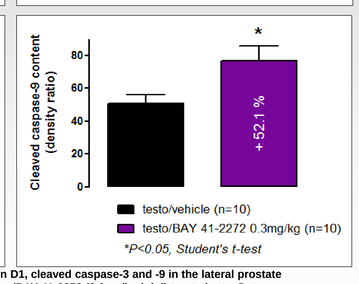
Evaluation of prostate enlargement by weighing of the gland. Data are mean ± SEM. p=ns, one-way ANOVA.

➡ **Neither vardenafil nor BAY 41-2272 modified prostate weight in testosterone-treated rats.**









Densitometric analysis of immunoblots for PCNA, cyclin D1, cleaved caspase-3 and -9 in the lateral prostate homogenates from testosterone/vehicle and testosterone/BAY 41-2272 (0.3mg/kg b.i.d) treated rats. Data are mean ± SEM. *p<0,05, Student's *t*-test.

➡ **Chronic administration of BAY 41-2272 (0.3 mg/kg b.i.d) significantly increased both cleaved caspase-3 and -9 expression, markers of apoptosis, and decreased, albeit not significantly, PCNA and cyclin D1 expression, markers of proliferation, thus favoring prostate regression.**

CONCLUSIONS

- **In addition to a potential relaxant effect on prostate smooth muscle fibers (dynamic component), sGC stimulation might favor prostate regression (static component).**
- **Of note, the effects of sGC stimulation on urethral pressure and voiding efficiency are still unknown.**
- **sGC stimulation could thus represent a novel promising pharmacological mechanism of action for the treatment of LUTS related to BPH.**