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This work was supported by a research grant from PFIZER

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ABSTRACT

Introduction and Objectives: Despite broad clinical use and significant efficacy of sildenafil for the treatment of erectile dysfunction (ED), certain patients with severe ED are poor responders. Our hypothesis is that chronic treatment could help salvage them to sildenafil therapy.

Methods: This study assessed the effects of an 8-week long chronic treatment with sildenafil (60 mg/kg/day SC 3x/day which gave a mean free plasma concentration of 39.7 ± 3.4 nM, n=22) or vehicle (n=22) in Sprague Dawley rats on intracavernosal pressure increase (expressed as ICP/MAP) elicited by electrical stimulation of the cavernous nerve and on endothelium-dependent relaxations of erectile tissue by *in vitro* isometric tension studies. The experiments were performed after a 36-hour wash-out period.

Results: No statistical difference was observed in the mean arterial pressure of the treated or untreated anaesthetized rats (74.7 ± 3.9 vs 71.5 ± 4.7 mmHg, NS). Chronic administration of sildenafil significantly improved and shifted to the left the frequency-dependent erectile response curve (responses to 10 Hz-6V were 85.0 ± 2.9 % vs. 71.1 ± 7.9 %, p<0.001). While contractile responses to phenylephrine 10⁻⁵ M were unchanged (192.4 ± 16.0 vs 152.7 ± 15.0 g/g ww, NS), relaxations to acetylcholine were enhanced in treated compared to untreated rats (10⁻⁵ M: -18.3 ± 3.6 % vs -13.6 ± 2.0, p<0.05, Two-Way ANOVA) without a significant change in pD₂ (-6.42 ± 0.16 vs -6.10 ± 0.22, NS). Conversely, relaxations elicited by A23187, a calcium ionophore, or by sodium nitroprusside were unaffected.

Conclusions: A chronic treatment with sildenafil enhances erectile responses in anaesthetized rats. The underlying increased endothelium-dependent corporal relaxations may be due to an upregulation of either muscarinic receptors or the transduction pathway leading to the activation of endothelial NOS. These experiments rule out an effect of chronic treatment with sildenafil on NO synthesis/breakdown or on the subsequent guanylate cyclase pathway, thereby eliminating a possible concern for tachyphylaxis. This could benefit patients with cardiovascular disease-related ED where corporal endothelial dysfunction occurs.

BACKGROUND

Although sildenafil has proven to be effective in treating ED, a report has suggested that its prolonged use may produce tachyphylaxis

El Galley et al., J. Urol. 2001
Lin et al., J. Urol. 2003

BUT, large clinical trials including patients for long periods of time have demonstrated only very small patient dropout rates for lack of efficacy

Carson et al., Urology 2002
Sadovsky et al., Int. J. Clin. Pract. 2001
Christiansen et al., Int. J. Impot. Res. 2000

⇒ Whether chronic treatment with sildenafil leads to tachyphylaxis is important to ascertain since it may have widespread implications, not only for sildenafil, but also for the other PDE-5 inhibitors if this is a class effect.

Moreover, despite broad clinical use and significant efficacy of sildenafil in the treatment of ED, certain patients with severe ED i.e. Erectile Function Domain Score from the IIEF below 11 remain poor responders

Sildenafil could have additional and prolonged beneficial effects on endothelial function in diabetic patients if taken on a daily basis

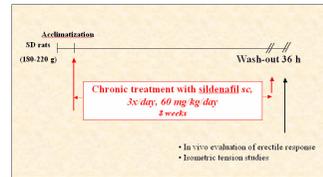
Desouza et al., Diabetes Care 2002

⇒ Chronic treatment, far from inducing tachyphylaxis, could help salvage non-responders to sildenafil therapy

OBJECTIVES

- ✓ To study the effects of **chronic treatment with sildenafil on the erectile responses** elicited by electrical cavernous nerve stimulation in anaesthetized rats
- ✓ To investigate erectile responses before and after an **acute administration of sildenafil in the chronically-sildenafil treated rats**
- ✓ To evaluate the effects of **chronic treatment with sildenafil on cavernosal tissue reactivity** by *in vitro* isometric tension studies

METHODS

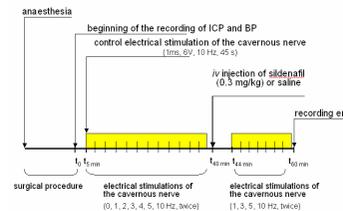
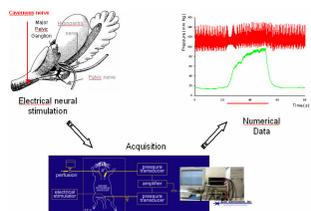


- Male Sprague-Dawley rats (180-220 g, n=44)
- Each experimental group received 20 mg/kg of sildenafil mesylate (or saline solution) via subcutaneous injection repeated three times a day (60 mg/kg/day in total) during 8 weeks
- Blood samples were collected before treatment, during the treatment period (bi-monthly, 6-8 h after a subcutaneous injection) and at the time of the *in vivo* experiments for the determination of free plasma concentrations of sildenafil (UK-92,480) and its active metabolite (UK-103,320)
- All experiments were performed after a 36-h wash-out period

In vivo evaluation of erectile function

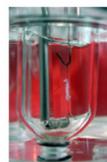
- Anaesthesia: urethane (1.2 mg/kg)
- Evaluation of erectile function by simultaneous monitoring of the arterial pressure and intracavernosal pressure (ICP) following electrical stimulation of the cavernous nerve *in vivo* (square-wave pulses of 1 ms, duration of 45 s, 6 V at different frequencies (0-10 Hz) performed in a randomized manner and repeated twice in order to establish frequency-response curves
- After the first train of stimulation, an intravenous injection of sildenafil 0.3 mg/kg was given and electrical stimulations of the cavernous nerve at 1, 3, 5 and 10 Hz were again performed 4 min after the injection

Results are expressed as the increase in ICP following electrical stimulation, normalized by the mean arterial pressure (MAP) of the animal



In vitro isometric tension studies

- Dissection of strips of rat corpus cavernosum to be studied in organ baths chamber



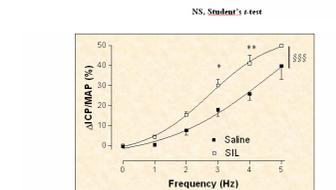
Pharmacological stimulation of

- endothelium-dependent relaxations concentration-response curves to acetylcholine and A23187, a calcium ionophore
- endothelium-independent relaxations concentration-response curves to sodium nitroprusside

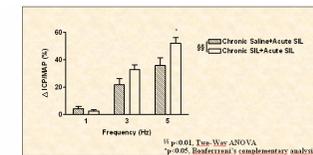
RESULTS

	Saline	Sildenafil
Beginning of study	243 ± 5	247 ± 7
End of chronic treatment	455 ± 9	451 ± 11

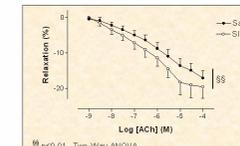
Mean arterial pressure under anaesthesia (mmHg)	
Saline	Sildenafil
71.5 ± 4.7	74.7 ± 3.9



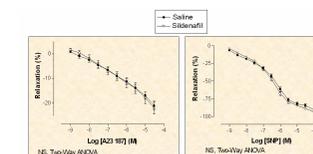
Chronic administration of sildenafil significantly enhanced frequency-dependent erectile responses in treated rats compared to untreated rats



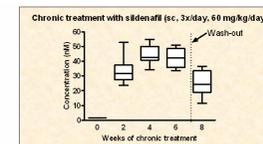
Rats treated with chronic sildenafil gave a greater response (45 % increase in the erectile response following electrical stimulation) to an acute injection of sildenafil compared to rats that had received saline chronically



Endothelial relaxation of cavernosal strips elicited by ACh was significantly enhanced (by 35 %) in rats treated chronically with sildenafil compared to untreated rats without a significant change in pD₂ (-6.42 ± 0.16 vs -6.10 ± 0.22, NS)



Relaxations elicited by A23187, a calcium ionophore, or by SNP were similar in tissues from chronically saline- or sildenafil-treated animals



Mean trough plasma concentrations reached concentration above 30 nM throughout the study
No detectable levels of drug or metabolite in untreated rats
After a wash-out period of 36 h, concentrations of free sildenafil and its metabolite were still detected in the plasma

Wash-out period was not sufficient to insure total elimination of sildenafil from the plasma of the chronically-treated rats

It is not possible to conclude that the enhancement of increases in ICP induced by cavernous nerve stimulation was solely imputable to the chronic treatment in the *in vivo* experiments,

NONETHELESS, for the *in vitro* experiments, tissue samples undergo extensive washings during the stabilization period before the experiments begin, thus removing residual compound from the tissue

In vitro experiments support the concept of profound tissue modifications following chronic treatment with sildenafil

CONCLUSION

Far from inducing tachyphylaxis, a chronic treatment with sildenafil:

- enhances erectile responses in anaesthetized rats
- sensitizes the chronically-treated rats to an acute administration of sildenafil
- potentiates ACh-induced endothelium-dependent cavernosal responses

Exposure to sildenafil obtained with 3x20 mg/kg/day sc closely related to those attained with a single therapeutic dose of 100 mg in humans (Nichols et al, 2002; Walker et al, 1999)

Chronic treatment with PDE-5 inhibitor could exert beneficial effects that go beyond acute PDE-5 inhibition i.e. functional tissular modifications involving the upregulation of muscarinic receptors or the transduction mechanisms leading to the activation of endothelial NOS

Chronic treatment with PDE-5 inhibitors could be of particular benefit in patients with cardiovascular disease-related ED where endothelial dysfunction occurs
⇒ Class effect ?

If confirmed in humans, this could help salvage poor responders to sildenafil therapy.