EXACERBATED PRESSOR RESPONSE REGULATED BY DAILY TREATMENT WITH SILDENAFIL IN AN ANIMAL MODEL OF METABOLIC SYNDROME

Behr-Roussel D.[1], Oudot A.[1], Compagnie S.[1], Gorny D.[1], Le Coz O.[1], Bernabé J.[1], Wayman C.[2], Alexandre L.[1], Giuliano F.[1, 3]

[1] *Pelvipharm, Domaine CNRS, 1 avenue de la terrasse, Bâtiment 5, F-91190 Gif-sur-Yvette, France – www.pelvipharm.com*

[2] *Pfizer Global Research and Development, Ramsgate Rd, Sandwich, Kent CT13 9NJ, UK*

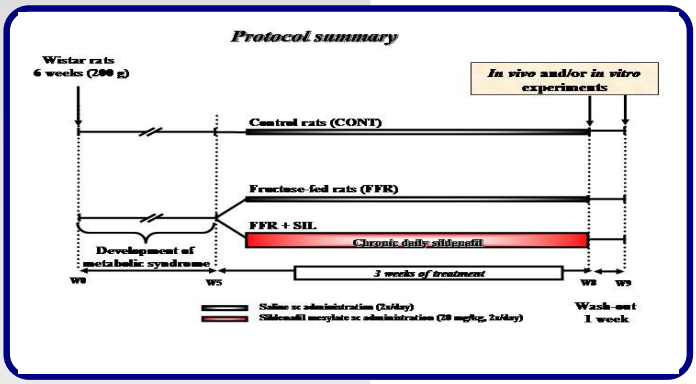
[3] *AP-HP Raymond Poincaré hospital, Department of Neurological rehabilitation, 104 Bd R. Poincaré, F-92380 Garches, France - giuliano@cyber-sante.org*

OBJECTIVES

- Fructose consumption might be a contributing factor to the development of obesity and the accompanying cardiovascular disorders (hypertension, …) seen in the metabolic syndrome
  Bray et al., Am J Clin Nutr (2004); Elliott et al., Am J Clin Nutr (2002); Hallfrisch, FASEB J (1990)
- Patients with metabolic syndrome exhibit generalized endothelial dysfunction
  Baron et al., Am J Cardiol (1999); Anderson et al., J Am Coll Cardiol (1995)
- Daily treatment with PDE-5 inhibitors has beneficial effects on endothelial function in diabetic men
  DeSouza et al., Diabetes Care (2002); Rosano et al., Eur Urol. (2005)

We postulated that chronic sildenafil in an experimental model of metabolic syndrome, the fructose-fed rat, could ameliorate arterial pressure regulation and biological surrogate markers of endothelial function such as (1) urinary 8-isoprostanes (IPT) content, a direct marker of non-enzymatic in vivo lipid peroxydation and the most reliable and clinically relevant marker of oxidative stress available to date, 2) urinary levels of TxB_2, the stable metabolite of TxA_2, a very potent vasoconstrictor.
Tissular content of (3) cGMP and (4) ET-1 in vascular segments were also evaluated.

MATERIALS & METHODS



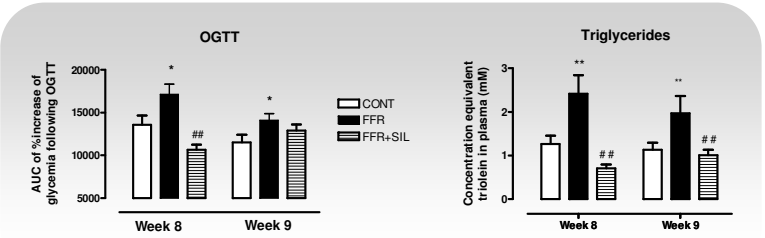
Experimental animals
Wistar rats (n=10-14 per group) were fed a standard chow (CONT) or a 60% fructose/5% fat (% by weight)-enriched diet for 8 weeks (FFR). From week 5 through 8, sildenafil was administered twice a day (sc, 20 mg/kg, FFR+SIL), thus reaching clinically relevant plasma concentrations *circa* 20 nM unbound known to give efficacy in man (*Pfizer Inc., data on file*), then a 1-week wash-out period from sildenafil was observed.

In vivo basal blood pressure/heart rate and pressor response to norepinephrine (NE) in conscious unrestrained rats
A sub-group of these rats (n=4-8 per experimental group) were implanted with a radio-telemetry transmitter (model TA11PA-C40, Data Sciences International, St. Paul, MN, USA) in the abdominal cavity under aseptic conditions using a femoral incision and under inhaled anaesthesia (2% isoflurane). The animals were allowed to recover during the wash-out period at least one week after surgery before blood pressure monitoring. Rats were allowed to acclimate to their new environment for 30 min before online recording of blood pressure for 30 minutes. Resting systolic, diastolic and mean arterial pressure were thus determined. Subsequently, increasing dosings of NE was infused for 5 minutes each (50, 100, 200, 400 ng/kg/min) and pressor responses determined for each dose as an average of the recorded response during the final minute.

Monitoring of endothelial biomarkerss
• At the end of the treatment period (week 8) as well as after the one-week wash-out period from the treatment (week 9), blood samples were taken after a 5-hr fasting period from the tail vein, plasma were separated and stored at –80°C where applicable. Determination of plasma glucose was performed by using a portable blood glucose meter on whole blood (Accu-chek active, Roche diagnostics, France).
•To perform oral glucose tolerance test (OGTT), rats were fasted overnight. The following morning, rats were gavaged with a solution of glucose 1 g/kg. Blood samples were taken at 0, 10, 20, 30, 60 and 90 minutes after the gavage and determination of plasma glucose was immediately performed. The results were expressed as the percentage of increase in glycemia compared to the basal level for each rat and the total area under the curve (AUC) was calculated.
• Triglyderides concentration was measured using a commercialized enzymatic colorimetric assay kit on plasma samples (Sigma, St Louis, MO, USA).
•For the determination of urinary IPT and TxB_2 content on 24-hour urine samples, rats were fasted overnight and placed in metabolic cages at the end of the one-week wash-out period. Immunoenzymatic determination of IPT and TxB_2 were performed according to the manufacturer's instructions (Cayman chemical, MI, USA). Urinary IPT and TxB_2 levels were corrected by the clearance of creatinine to limit variability in the assays due to changes in renal excretory function.
•After BP evaluation, rats were deeply anesthetized with an intraperitoneal injection of urethane (1.2 g/kg). Tissue samples (thoracic aorta and superior mesenteric artery) were harvested, immediately frozen in liquid nitrogen, and stored at –80°C until assessment of cGMP and ET-1 tissular content.
Tissue samples for cGMP determination were homogenized in a glass potter at 4°C in a fixed volume of phosphate/theophylline buffer (phosphate buffer 50 mM ; theophylline 1 mM; pH 7.4), centrifuged and the supernatants assayed for protein and cGMP. The quantitation of tissular cGMP was performed using a commercially available cGMP enzyme immunoassay kit (Cayman Chemical, MI, USA).
Extraction of ET-1 from blood vessels was performed using the method of Verma et al. (Am J Physiol, 1995). The supernatant was then passed through an Amprep C2 mini-column (Amersham, UK) and the eluant was reconstituted in 250 µl assay buffer from the commercial assay EIA kit that was used for subsequent determination of ET-1 concentrations (Cayman Chemical, MI, USA).

RESULTS

- **No influence of the fructose-enriched diet on the animal body weight or blood glucose levels**



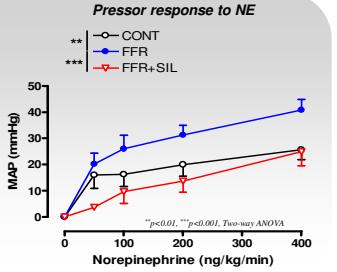
$^{*}p<0.05$, $^{**}p<0.01$ vs CONT, One-way ANOVA; $^{\#\#}p<0.01$ vs FFR, One-Way ANOVA

- **Impaired glucose tolerance in FFR corrected by a chronic treatment with sildenafil**
- **Chronic sildenafil treatment significantly countered the pronounced hypertriglyceridemia secondary to the fructose feeding in FFR and this effect was maintained even after the one-week wash-out period**

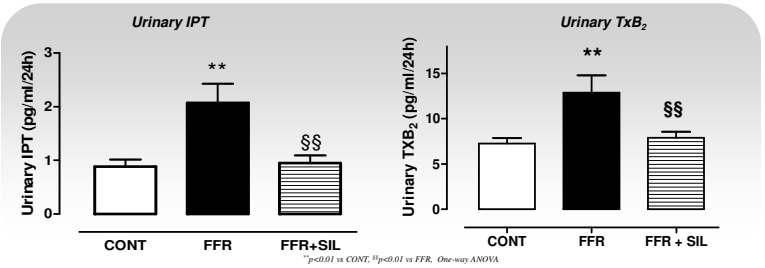
Baseline blood pressure and heart rate after one-week wash-out from daily sildenafil treatment

	CONT (n=8)	FFR (n=6)	FFR + SIL (n=4)
MAP (mmHg)	106.5 ± 4.5	103.2 ± 5.6	109.4 ± 8.8
Diastolic BP (mmHg)	95.6 ± 4.6	92.1 ± 5.0	96.8 ± 7.7
Systolic BP (mmHg)	120.1 ± 4.7	116.4 ± 6.2	124.3 ± 10.4
Heart Rate (bpm)	407 ± 19	380 ± 9	423 ± 9

NS, One-way ANOVA



- **Baseline BP and heart rate were unchanged by the fructose-enriched diet, and chronic sildenafil administration did not modify these parameters, during or after one-week washout from sildenafil therapy**
- **Chronic sildenafil administration corrected the exacerbated pressor response to NE caused by the fructose-enriched diet, even one week after treatment cessation**



- **Neither the fructose diet nor the sildenafil treatment modified significantly tissue basal cGMP and ET-1 content in homogenates of aortas and superior mesenteric arteries after one-week washout from daily sildenafil treatment**
- **Chronic treatment with sildenafil was able to restore normal levels of urinary IPT and TxB_2, even one week after cessation of the treatment**

CONCLUSIONS

- **Effect of chronic sildenafil treatment on physiological parameters relevant to the metabolic syndrome : Correction of the enhanced response to glucose overload, as well as the hypertriglyceridemia induced by fructose feeding**
- **Effect of chronic sildenafil treatment on exacerbated *in vivo* pressor response to NE in conscious unrestrained FFR: Restoration of normal blood pressure control in response to NE infusion**
- **Effect of chronic sildenafil treatment on urinary 8-isoprostanes and TxB_2 excretion : Normalization of the excretion of a biological marker for oxidative stress and a biological marker of a potent vasoconstrictor**

The beneficial effects of daily sildenafil on blood pressure regulation gives additional insight to the possible mechanism of action of sildenafil in cardiovascular disorders related to the metabolic syndrome that could be explored in future clinical trials.

Moreover, this study provides preclinical support for the predictive value of biological markers such as urinary IPT and TxB_2 as surrogate markers in future clinical trials addressing cardiovascular risks.

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