TOTUM-854 prevents L-NAME-induced arterial hypertension in mice

Gaetan BOLEA[(1)], Doriane RIPOCHE[(2)], Gregory MEYER[(1)], Florian LEJOUBIOUX[(3)], Arnaud MICHAUX[(2)], Yolanda F. OTERO[(2)], Sébastien L. PELTIER[(3)], Pascal SIRVENT[(2)], Cyril REBOUL[(1)]

[(1)]LAPEC EA-4278, Avignon Université, Avignon, France; [(2)]Valbiotis, R&D Riom Center, Riom, France; [(3)]Valbiotis, R&D Périgny Center, Périgny, France

CONTEXT

Arterial Hypertension (HTA) is a major cause of premature death worldwide, with an estimated global prevalence of 1.13 billion people. Polyphenolic compounds have been shown promising effects in the context of HTA management[1]. We have developed TOTUM-854 (T-854), a polyphenol-rich botanical composition to reduce the risk of developing AHT. The aim of this study was to assess the chronic effect of T-854 on preventing the development of N(G)-Nitro-L-arginine-methyl ester (L-NAME)-induced AHT in mice (Study 1). We also evaluated the acute effect of T-854 on blood pressure in spontaneous hypertensive rats (SHR) (Study 2).

METHOD

Study 1. Male C57Bl6/J mice (n=11 per group) received L-NAME (100 mg/kg/day) dissolved in drinking water and were administered either T-854 (500 mg/kg) or vehicle (1% Tween 20) once a day for three weeks by gavage (Table 1). Every week, body weight was measured, and arterial pressure was recorded using the CODA® Tail-Cuff System (Kent Scientific) after one-week acclimatization (Figure 1). All measurement were performed by the only one and same trained operator, and at the same time frame (from 9 am to 1 pm). Every weekly data were issued by the average of at least 7 measures (from 15 measuring cycles per machine)[2].

Table 1. Group description for study 1.

Group	Induction	Product administration	Animal n
Control	-	Vehicle	11
L-NAME	L-NAME	Vehicle	11
L-NAME +T-854	L-NAME	T-854 (500mg/kg)	11

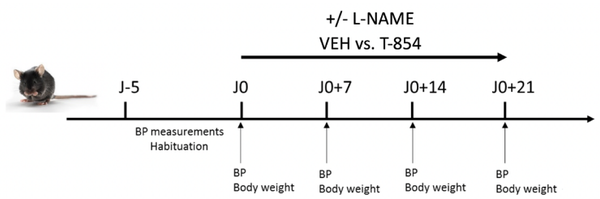


Figure 1. Experimental design (Study 1): Effects of chronic supplementation with T-854 (500 mg/kg) *vs* vehicle (VEH) on blood pressure in mice with L-NAME-induced HTA. BP. Blood Pressure.

Study 2. 12 week-old male SHR rats (n=12) were used to analyse acute effect of T-854 on arterial pressure. A surgery was performed to implant a radio-telemetry device (HD-S10, DSI) directly into the abdominal aorta under isoflurane anaesthesia. Rats were allowed to recover for at least 7 days. Animals received a dose of vehicle (1% Tween 20) and T-854 (1250 mg/kg) *per os* with a 48h-wash-out interval between two gavages, in random order (crossover design). Arterial pressure was recorded during 24h post-gavage. Baseline arterial pressure was measured before oral gavage during 1h30 (Figure 2).

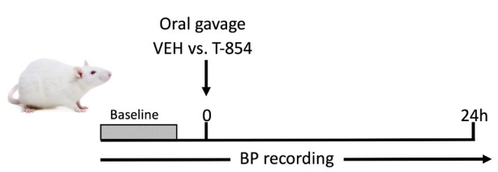


Figure 2. Experimental design (Study 2): Effects of acute supplementation with T-854 (1250 mg/kg) *vs* vehicle (VEH) on blood pressure in hypertensive SHR rats. BP. Blood Pressure.

Statistical analysis. Values were presented as the mean ± SEM. The differences were considered statistically significant at $p < 0.05$. Shapiro-Wilk normality test were used to determine whether the data are consistent with a Gaussian distribution. If data are not distributed according to the normal distribution, a Kruskal-Wallis nonparametric test were used followed by Dunn test for *post hoc* comparison. When normal distribution is assumed measures were subjected to one-way or two-way ANOVA with Tukey and Sidak tests for multiple comparison respectively.

RESULTS

1) L-NAME mice study

- Effect on body weight

No difference was observed in body weight evolution between the three groups (Figure 3).

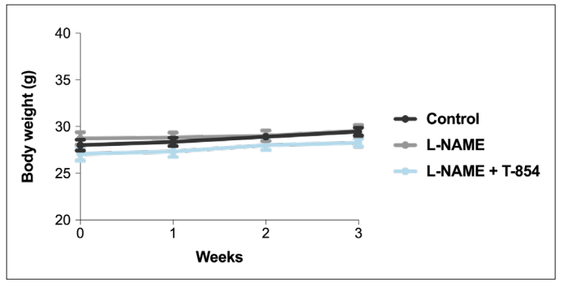


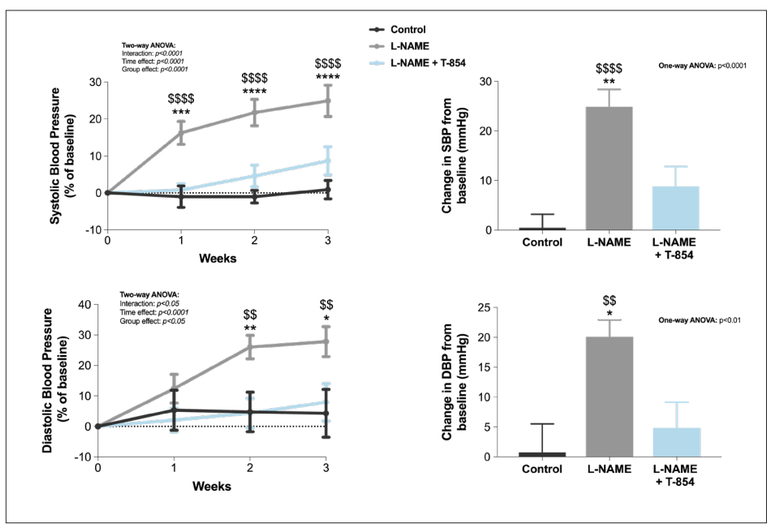
Figure 3. Effect of L-NAME and T-854 administration on body weight evolution along three study weeks.

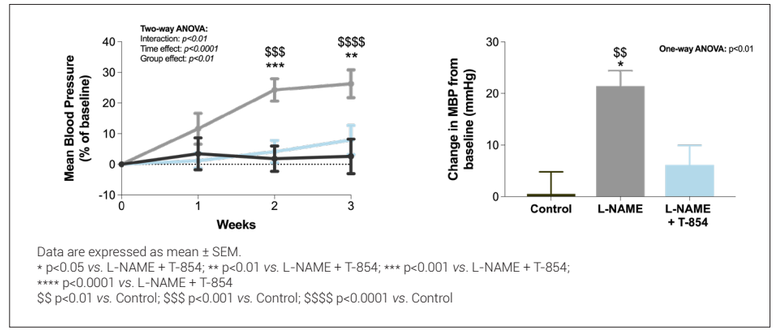
- Effect on blood pressure

L-NAME increased systolic (SBP; from 105 ± 3 to 130 ± 1 mmHg, p<0.0001), diastolic (DBP; from 77 ± 3 to 97 ± 2 mmHg, p<0.0001) and mean (MBP; from 86 ± 3 to 108 ± 2 mmHg, p<0.0001) blood pressure during the 3 weeks of treatment (Figure 4). Interestingly, in T-854-supplemented mice, SBP was significantly reduced from the first week of supplementation and DBP and MDP from the second week of supplementation.

After three weeks of supplementation, T-854 lowered SBP by 16 mmHg (p<0.0001), DBP by 15 mmHg (p<0.05) and MBP by 15 mmHg (p<0.01), in comparison to non-supplemented L-NAME-treated mice (Figure 4).

No difference was observed between L-NAME + T-854 and Control group, showing the abolition of L-NAME-induced BP elevation by T-854.





Data are expressed as mean ± SEM.
* p<0.05 *vs.* L-NAME + T-854; ** p<0.01 *vs.* L-NAME + T-854; *** p<0.001 *vs.* L-NAME + T-854;
**** p<0.0001 *vs.* L-NAME + T-854
$$ p<0.01 *vs.* Control; $$$ p<0.001 *vs.* Control; $$$$ p<0.0001 *vs.* Control

Figure 4. Effect of L-NAME and T-854 administration on blood pressure. Left panels represent blood pressure percentage of baseline along three study weeks. Right panels represent final change from baseline in blood pressure after three weeks of L-NAME and T-854 administration. SBP. Systolic Blood Pressure; DBP. Diastolic Blood Pressure; MBP. Mean Blood Pressure.

2) Spontaneous Hypertensive Rats study

SHR rats exhibited an elevated SBP (164.4 mmHg ± 4.7 mmHg) and DBP baseline (115.9 mmHg ± 3.6 mmHg), validating hypertensive status in animals (Table 2).

24h-area under curve for SBP and DBP was calculated for each rat, gavaged in vehicle and T-854. T-854 response was normalized to vehicle response for each rat.

After T-854 gavage, SBP and DBP were reduced during 24h recording in comparison to vehicle, with a 24h-AUC decrease by 108 ± 87.8 and 84.4 ± 69.3 respectively (Table 2).

Table 2. Baseline and 24h-T-854 AUC decrease, normalized to vehicle in 12 w-old SHR rats.

	Baseline (mmHg, mean ± SEM)	24h-T-854 AUC decrease, normalized to vehicle (mean ± SEM)
SBP	164.4 ± 4.7	-108.0 ± 87.8
DBP	115.9 ± 3.6	-84.4 ± 69.3

CONCLUSION

T-854 prevents the development of HTA, after three weeks of L-NAME in mice. Its 24h-post-gavage acute effect on blood pressure in hypertensive SHR rats suggests a rapid effect, with a decrease in blood pressure just few hours after oral administration and maintained during the time of the experiment.

In conclusion, T-854 appears as an efficient strategy to prevent HTA and this effect was confirmed in two different preclinical models: L-NAME-induced HTA in mice and SHR rats.

Further studies are presently conducted to elucidate the mechanisms of action associated with the beneficial effects of T-854 on blood pressure management. These results open the door to clinical studies conducted in hypertensive subjects.

[1]Hügel H. M. et al. Phytomedicine. 2016
[2]Battault S. et al. Am J Physiol Heart Circ Physiol. 2018