Title:
Overview of nonclinical studies of LIB-01, a novel promising oral drug under development for treatment of erectile dysfunction

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Introduction:
LIB-01 is a novel molecule being developed as an oral drug for the treatment of erectile dysfunction (ED). It is a semi-synthetic analog, developed based on research on active compounds identified in root bark that had been used for treatment of male sexual disability since time immemorial.
Objective:
In preparation for clinical development, a number of nonclinical studies on erectile function were conducted in normal and diabetic rats with ED. Studies to investigate the mechanism supporting the pro-erectile effect of LIB-01 were also conducted. A summary of the results from the nonclinical research program is presented.
Methods:
Erectile response elicited by electrical stimulation of the cavernous nerve (ES CN) in anaesthetized rats was assessed. Intracavernous and arterial blood pressures were monitored according to a standardized experimental procedure. Mechanism of action studies were conducted with rat cavernosal tissue samples using immunohistochemistry and in *ex vivo*/organ chamber experiments. The isometric tension studies were conducted on cavernosal strips from LIB-01 treated and untreated Wistar rats. Cavernosal strips from untreated rats were incubated with either LIB-01 or DMSO (control) and then pre-contracted by phenylephrine (PHE). Concentration- or frequency response curves were performed on PHE pre-contracted strips in 3 consecutive steps: (i) cumulative addition of increasing acetylcholine (ACh) concentrations, (ii) electrical field stimulation (EFS) with increasing frequencies, and (iii), cumulative addition of increasing sodium nitroprusside (SNP) concentrations.
Results:
A statistically significant pro-erectile facilitatory effect of LIB-01 has been repeatedly evidenced in all the studies conducted in anaesthetized control Wistar rats, by oral or subcutaneous administration of LIB-01 at 15 mg/kg/d, with an effect in the similar range as *iv* sildenafil 0.3mg/kg, when compared. The pro-erectile effect of LIB-01 was not acute, instead, the effect gradually increased and remained for at least 7 days post treatment. In addition, LIB-01 significantly improved erectile responses in anaesthetized diabetic Goto-Kakizaki ED rats compared to vehicle-treated rats, demonstrating the pro-erectile effect of LIB-01 in a robust validated pathophysiological rat model of type 2 diabetes.
Immunohistochemistry experiments on cavernosal tissue from LIB-01 treated normal rats showed that neither CD31 nor nNOS expression was altered, thus likely ruling out any effect

of LIB-01 on cavernosal micro-vascularization or cavernosal nNOS. Furthermore, only at a high concentration (10^{-5} M) was there a slight increase in the endothelium dependent relaxations induced by ACh compared to vehicle while LIB-01 did not exert any effect neither on endothelium-independent relaxations induced by SNP nor on nitrergic relaxation induced by EFS of untreated cavernosal strips exposed *ex-vivo* to different concentrations of LIB-01.

Conclusions:

LIB-01 is a novel molecule which displays a significant pro-erectile effect in anesthetized normal rats as well as in diabetic rats with ED. The pro-erectile effect of LIB-01 is not acute, instead, the effect gradually increases and remains for at least 7 days post-treatment. Results from several mechanistic studies lead to postulate that the mechanism of LIB-01 does not recruit the NO-cGMP pathway thereby differing from phosphodiesterase type 5 inhibitors. A first-in-human clinical trial with LIB-01 is planned to start in 2023.