Diabetic Complications Consortium

Application Title: Cathepsin S inhibition and diabetic neuropathy

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1. Project Accomplishments:

We investigated the efficacy of cathepsin S inhibition on behavioral, functional and structural indices of peripheral neuropathy in a mouse model of type 1 diabetes. Cathepsin S was inhibited either from onset of diabetes (prevention paradigm) or after neuropathy was detected (intervention paradigm). The major findings were:

1. Cathepsin S inhibition attenuated large sensory fiber mediated tactile allodynia in diabetic mice when measured 24hr after last treatment.
2. Cathepsin S inhibition reversed large fiber motor nerve conduction slowing in diabetic mice in the absence of any diabetes-induced reduction in axonal diameter.
3. Cathepsin S inhibition was without effect on reduced levels of myelin proteins MBP and P0 in the sciatic nerve of diabetic mice.
4. Cathepsin S inhibition was without effect on loss of small sensory fiber mediated paw heat sensation in diabetic mice or depletion of epidermal and dermal fibers of diabetic mice.

Our data suggests that cathepsin S inhibition primarily targets large myelinated fiber neuropathy in diabetes but that efficacy is not associated with protection of axon diameter or myelin components. Additional studies are in progress.

2. Specific Aims:

Specific Aim: The Specific Aim was to determine the potential efficacy of cathepsin S inhibition on behavioral, functional and structural indices of degenerative neuropathy in a rat model of type 1 diabetes.

Modification to Specific Aim*:* Unavailability of the rat-specific cathepsin S inhibitor (VBY285) caused us to switch to the STZ-diabetic mouse model of type 1 diabetes, as mouse-specific inhibitors (VBY129 and VBY036) were available and we had preliminary data showing equivalent efficacy to the rat inhibitor in a model of chemotherapy induced neuropathy that was presented in the initial application. STZ-diabetic rats and mice display a similar neuropathy phenotype and the reduced cost associated with mouse studies allowed us to modify the experimental design to incorporate two separate studies (prevention or reversal therapy) rather than the single combined prevention/reversal study originally proposed. This enhanced statistical power. However, the reduced tissue volume available from mice caused us to alter some of the neuropathy assays.

Results:

1. Systemic diabetes

Treatment with the cathepsin S inhibitors VBY129 or VBY036 did not alter systemic indices of diabetes compared to vehicle treated diabetic mice (**Table 1**).

GROUP	*N*	*Body Weight (g) at onset of treatment*	*Body Weight (g) at end of study*	*Blood Glucose (mg/dl) at end of study*
Prevention study				
Control+Vehicle	9	24.0±0.4	30.6±1.4	11.6±0.5
Control+VBY129	10	24.1±0.3	29.9±1.0	11.4±1.0
Diabetic+Vehicle	6	25.1±0.5	29.0±0.4	43.4±4.4
Diabetic+VBY129	7	25.6±0.6	28.4±1.0	48.6±0.8
Reversal study				
Control+Vehicle	8	30.4±1.3	30.5±1.6	9.9±0.9
Diabetic+Vehicle	8	28.2±1.0	28.5±1.2	32.4±5.9
Diabetic+VBY036	8	26.8±1.3	27.5±1.3	40.2±4.1

2. Large sensory fiber function.

Preliminary data shown in the application indicated that the cathepsin S inhibitor VBY036 both acutely alleviated tactile allodynia in a mouse model of paclitaxel-induced neuropathy and also produced a long-acting restoration of normal function following repeated treatment. We extended this work to STZ diabetic mice by measuring tactile sensitivity at regular intervals following onset of treatment with a cathepsin S inhibitor, with each measurement made 24 hr after the last treatment to avoid acute effects, which resolve in 4hr. Diabetes induced tactile allodynia was attenuated by VBY129 and VBY036 **(Fig. 1**). Future studies will examine the dose dependency of this prolonged effect of cathepsin S inhibition and investigate whether it reflects altered drug PK/PD over time or a change in neuropathy phenotype independent of immediate drug levels at the target site.

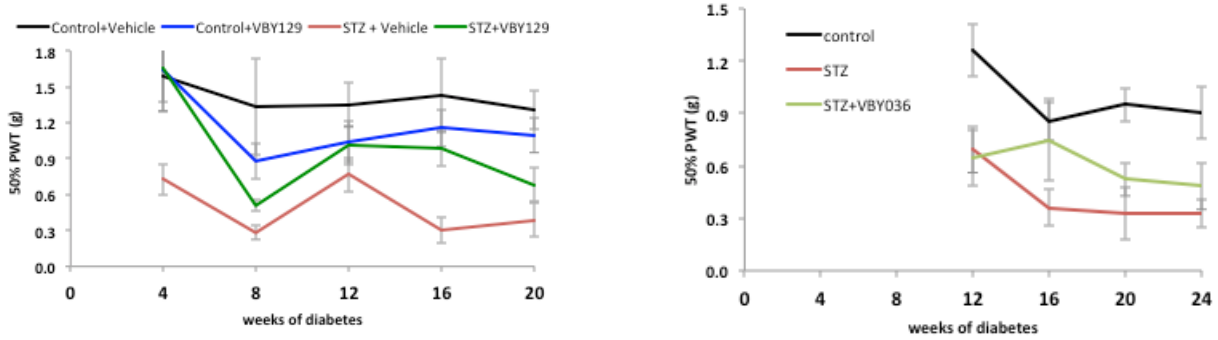


Fig. 1. Paw sensitivity to von Frey filaments, quantified as 50% paw withdrawal threshold (PWT) in control and STZ-diabetic Swiss Webster mice treated with vehicle or with the cathepsin S inhibitors VBY129 (10mg/kg/day s.c.) from onset of diabetes (left panel) and VBY036 (100mg/kg/day s.c.) starting after 12 weeks of untreated diabetes (right panel). Data are group mean±SEM of N=6=10/group.

3. Large motor fiber function

Diabetes caused a reduction in motor nerve conduction velocity (MNCV) in the sciatic nerve (**Fig. 2**). Treatment with VBY129 (10mg/kg/day sc) from onset of diabetes caused improvement in MNCV of diabetes rats but only after 16+ weeks of treatment. More strikingly, impaired MNCV was completely reversed by VBY036 (100 mg/kg/day sc) when given following 12 weeks of untreated diabetes.

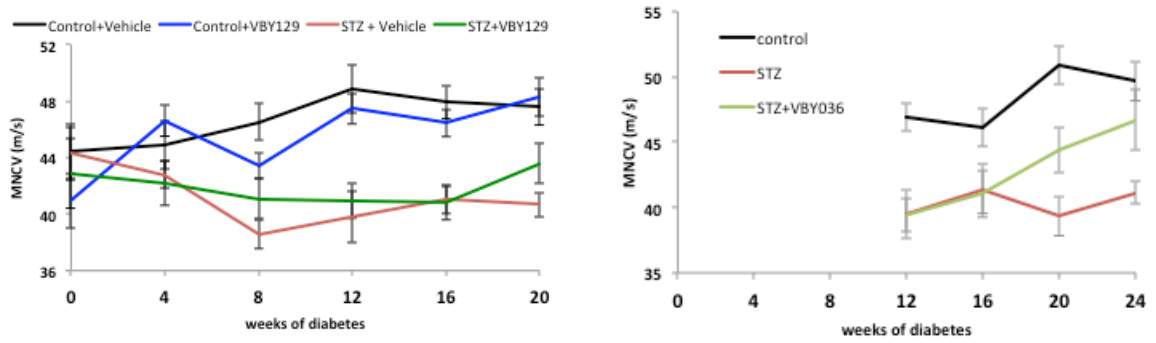


Fig. 2. Sciatic MNCV (m/s) in control and STZ-diabetic Swiss Webster mice treated with vehicle or with the cathepsin S inhibitors VBY129 (10mg/kg/day s.c.) from onset of diabetes (left panel) and VBY036 (100mg/kg/day s.c.) starting after 12 weeks of untreated diabetes (right panel). Data are group mean±SEM of N=6=10/group.

4. Large fiber structure

Peripheral nerve was processed to resin blocks and cut for light microscopy to allow measurement of axonal diameter of large myelinated fibers. We have previously reported reduced mean axonal diameter in STZ-diabetic rats, but prior studies for Diacomp showed no decrease in mean axonal caliber in assorted mouse models of diabetes (Calcutt, unpublished RAID program studies). An initial analysis showed no significant change in mean axonal diameter or the axonal diameter distribution between control and STZ-diabetic mice. MNCV slowing in diabetic mice (**Fig. 2**) is therefore not related to axonal atrophy and further analyses were aborted.

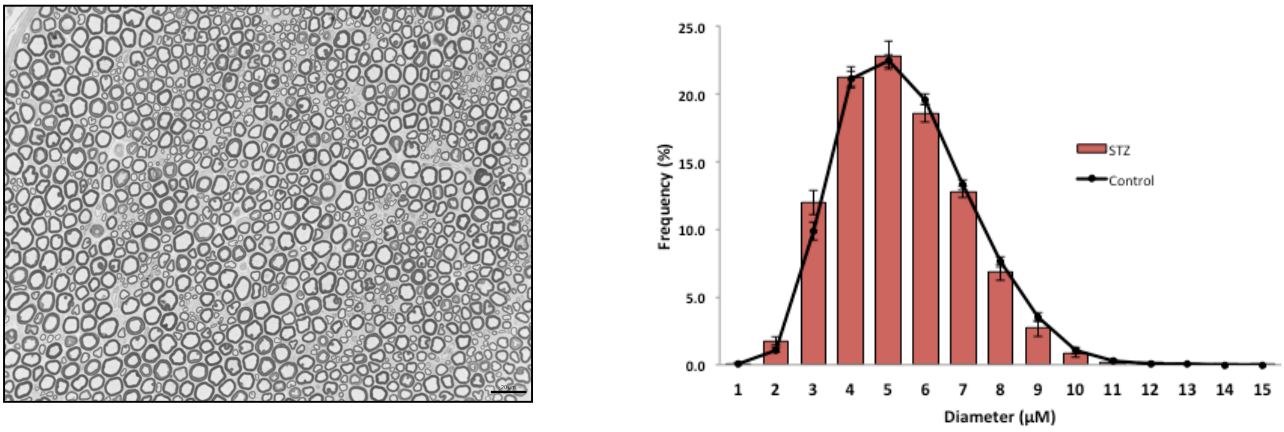


Fig. 3. LEFT: Representative image of mouse peripheral nerve prepared for axonal morphometry (bar = 20μm). RIGHT: Myelinated fiber axonal size:frequency distribution in peripheral nerve of control and STZ-diabetic Swiss Webster mice. Data are group mean ± SEM of N=8/group.

5. Small sensory fiber function

Paw heat-evoked withdrawal response temperature increased in STZ-diabetic mice, indicative of impaired small fiber nociceptive function. Cathepsin S inhibition did not alter thermal hypoalgesia in either preventative or reversal paradigms (**Fig. 4**). It was notable that paw thermal hypoalgesia of diabetic mice treated with vehicle or VBY129 diminished over time in the reversal study (right panel), suggesting spontaneous recovery of function.

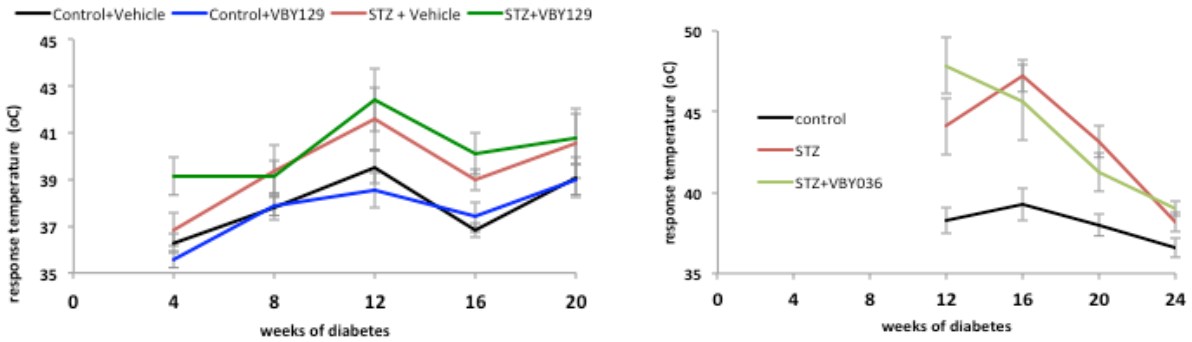
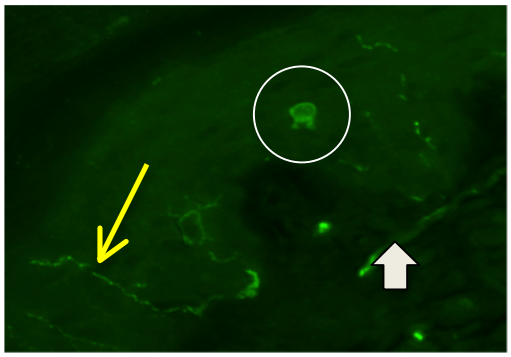


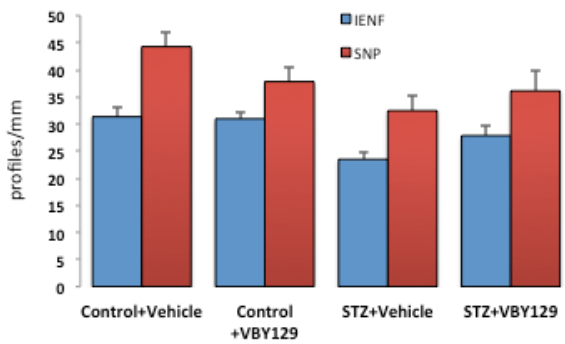
Fig. 4. Paw withdrawal temperature (°C) in control and STZ-diabetic Swiss Webster mice treated with vehicle or with the cathepsin S inhibitors VBY129 (10mg/kg/day s.c.) from onset of diabetes (left panel) and VBY036 (100mg/kg/day s.c.) starting after 12 weeks of untreated diabetes (right panel). Data are group mean±SEM of N=6=10/group.

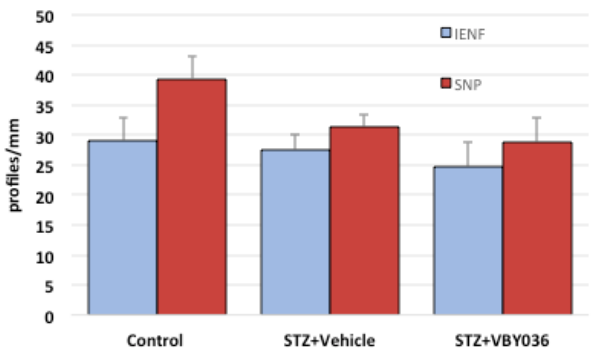
6. Small sensory fiber structure

Paw skin was processed for visualization of small sensory fibers in the epidermis (IENF) and dermis (SNP) using anti-PGP9.5 antibody (**Fig. 5**). In the prevention study, 20 weeks of diabetes caused loss of IENF and SNP, whereas in the reversal study, there was depletion of SNP but not IENF by week 24 of diabetes. This is consistent with gradual diminution of thermal hypoalgesia (**Fig. 4.**) and suggestive of collateral sprouting of IENF terminals at later stages of diabetes. Treatment with either VBY129 or VBY036 was without marked effects.

Fig. 5. UPPER LEFT PANEL: Representative image of mouse paw skin immunostained with anti-PGP9.5 antibody and illustrating dermal nerve (SNP: white arrowhead), epidermal nerve (IENF: yellow arrow) and Langerhans cells (white circle). LOWER PANELS: IENF and SNP density in paw skin of control and STZ-diabetic Swiss Webster mice treated with vehicle or the cathepsin S inhibitor VBY129 (10mg/kg/day s.c.) from onset of diabetes or VBY036 (100mg/kg/day s.c.) starting after 12 weeks of untreated diabetes (right panel. Data are group mean±SEM of N=6=10/group.







7. Nerve myelin proteins

The striking effect of cathepsin S inhibition in reversing MNCV slowing in diabetic mice **(Fig. 2.** right panel) in the absence of any change in axonal diameter **(Fig. 3**.) suggested an exploratory focus on myelin proteins associated with large myelinated fibers in nerve from these animals. Western blots for the myelin proteins PO and MBP demonstrated reduced sciatic nerve content in tissue from diabetic mice that was not altered in mice treated with VBY036 for the last 12 weeks of a 24 week period of diabetes (**Fig. 6**).

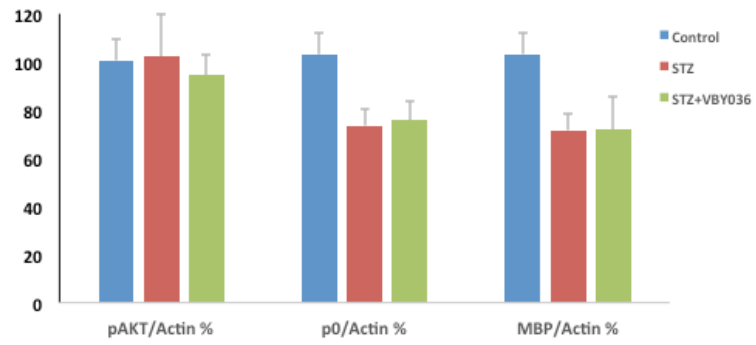


Fig. 6. Densitometric quantification of western blots for pAKT, PO and MBP normalized to actin in sciatic nerve from control and STZ-diabetic Swiss Webster mice treated with vehicle or with the cathepsin S inhibitor VBY036 (100mg/kg/day s.c.) starting after 12 weeks of untreated diabetes (right panel). Data are group mean±SEM of N=5-6/group.

8. Work in Progress

i) Quantification of corneal nerve density in images collected by corneal confocal microscopy.
ii) We have residual sciatic nerve homogenates for selected western blotting.

3. Publications:

None as yet – an embargo on release of data to the public is requested to allow us to prepare and submit a manuscript that includes data produced by this project.