Selective Toxicity of the Anthelmintic Emodepside Revealed by Heterologous Expression of Human KCNMA1 in *Caenorhabditis elegans*

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Molecular Pharmacology

Supplementary Information S1

RT PCR to confirm presence of *slo-1* and *kcnma1* in transgenic C*. elegans*.

Method

Total RNA was extracted from wild-type *N2*, *slo-1(js379)*, *slo-1(js379);p slo-1::slo-1* and *slo-1(js379);pslo-1::kcnma1 C. elegans*. Well fed mixed populations of *C. elegans* were washed off the plates with M9 buffer and allowed to settle at the bottom of the tubes. After discarding the supernatant the remaining worm pellets were further washed with M9 buffer twice to remove bacteria. Resultant worms were homogenised in a glass homogeniser with Trizol reagent for 10 minutes. After centrifuging for 30 minutes, 1/5 part of chloroform was added to the supernatant. This was vortexed and incubated at room temperature for 3 minutes. Colourless total RNA containing phase was transferred to new tubes after a 15 minutes centrifugation step. Isopropyl alcohol in a volume equal to the volume of the remaining supernatant was then added to precipitate RNA. This was left over night at 4° C or at room temperature for 10 minutes. The supernatant was discarded after a 10 minutes centrifugation step. The pellet was washed with 1ml of 75% ETOH/DEPC dH_2O. This was then centrifuged

for 15 minutes. After discarding the supernatant the pellet was air dried and resuspended in 30μl of DEPC dH_2O. All centrifugation steps were performed at 13,000 rpm at 4° C. The concentration and purity of the RNA was estimated by a NanoDrop ND-1000 Spectrophotometer. Samples were further treated with RNAse free DNAse I (QIAGEN) to remove remaining DNA. Total RNA was then reverse transcribed using iSCRIPT Select cDNA synthesis kit (BioRad). The procedure was performed in two steps.

In a first step cDNA was synthesised from 4μg of treated total RNA per sample. Oligo dTs were used to enrich for mRNA in a reaction. In negative controls reverse transcriptase was substituted with dH_2O. In a second step 2μl of cDNA per sample was amplified by PCR using *Taq* DNA polymerase (Roche). 15μl of DNA was removed from the PCR at cycles 25, 30 and 35. This was done in parallel for the test and control samples to distinguish between cDNA synthesised from mRNA and remaining plasmid DNA. 10ng of plasmid DNA or dH_2O were used as positive and negative controls for the PCR reaction. Alternatively, 10μl of cDNA per sample was amplified to verify the presence of *slo-1* DNA in *N2* and *slo-1(js379) C. elegans*. Primers for PCR were designed to anneal to the introns separated by exons of *slo-1* and *kcnma1* RNA to distinguish between mRNA and genomic DNA.

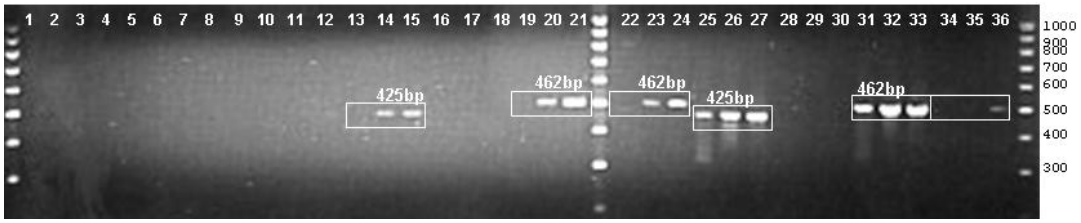
Primers for the second step of RT PCR:

slo-1 left	ATTTGGGCCAACAAGTTCAG
slo-1 right	CAGCGTCTTCCACTCTTTCC
kcnma1 left	GGAGGATGCCTCGAATATCA
kcnma1 right	GCATCACTTGCGATGAAAAA

Results and Discussion

The PCR products generated from total RNA extracted from transgenic worms is shown in Figure S1.1

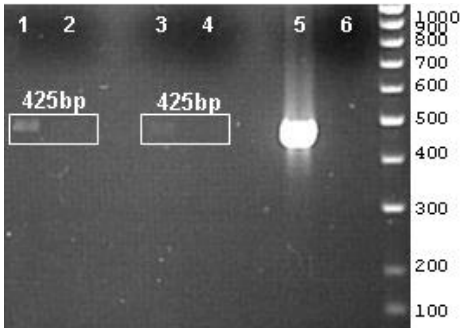
Figure S1.1 Amplification of cDNAs for *slo-1* and *kcnma1* from total RNA extracted from transgenic worms



15µl of DNA was separated on a 1.5% Agarose gel next to 100bp DNA ladder (GeneStar). Control samples for the reverse transcription in the first step of RT PCR contained dH_2O instead of reverse transcriptase. Positive controls for the PCR in the second step of RT PCR contained 10ng of plasmid DNA. Negative controls for PCR contained dH_2O instead of DNA. 15µl of samples were removed for analysis after 25, 30 and 35 cycles of PCR. Lanes 1-24 contained DNA reverse transcribed from 4µg of total RNA extracted from *C. elegans*. 2µl cDNA was used for the PCR in the second step of RT PCR. Lanes 1-3: *N2*; lanes 4-6: *N2* control; lanes 7-9: *slo-1(js379)*; lanes 10-12: *slo-1(js379)* control; lanes 13-15: *js379;pslo-1::slo-1*; lanes 16-18: *js379;pslo-1::slo-1* control; lanes 19-21: *js379;pslo-1::kcnma1*; lanes 21-24: *js379;pslo-1::kcnma1* control. Lanes 25-36 contained positive and negative PCR controls. Lanes 25-27: positive control for *slo-1*; lanes 28-30: negative control for *slo-1*; lanes 31-33: positive control for *kcnma1*; lanes 34-36: negative control for *kcnma1.* No expression of *slo-1* mRNA was identified in wild-type *N2 C. elegans* (lanes 1-3). This might be due to low endogenous expression of *slo-1* from genomic DNA. *Slo-1(js379)* mutants contain a

premature “STOP” codon in position 251 in the primary protein sequence, which corresponds to positions 751-753 in the ORF for *slo-1*. Although, SLO-1 will not form a functional channel in these mutants, the corresponding mRNA transcript should be present. Absence of a band corresponding to reverse transcribed *slo-1* in *slo-1(js379)* mutants (lanes 7-9) would suggest that *slo-1* mRNA is either completely absent or unstable. Low levels of expression might be another reason. *Slo-1* mRNA was detected in *js379;pslo-1::slo-1 C. elegans*, which corresponds to the bands of 425 base pairs after 30 and 35 cycles of PCR (lanes 14-15). No reverse transcribed *slo-1* was detected in control for reverse transcription (lanes 16-18) or negative control for PCR (lanes 28-30). Positive control for PCR contained bands of 425 base pairs, which correspond to 25, 30 and 35 cycles of PCR (lanes 25-27). *Kcnma1* mRNA was detected in *js379;pslo-1::kcnma1 C. elegans*, which corresponds to the bands of 462 base pairs after 30 and 35 cycles of PCR (lanes 20-21). Bands of lower intensity were detected in control for reverse transcription (lanes 23-24). This suggests that a part of detected *kcnma1* DNA in lanes 20-21 was amplified from plasmid DNA, which had not been completely removed by DNAse I treatment. The positive control for PCR (plasmid DNA) contained bands of 462 base pairs, which correspond to 25, 30 and 35 cycles of PCR (lanes 31-33). A faint band in lane 36 corresponding to the negative PCR control for *kcnma1* after 35 cycles points to the low levels of contamination of PCR products. The contamination was caused by plasmid DNA. Additional steps had been taken to remove contamination from the PCR, which reduced it to the levels observed here. To conclude, amplification products in lanes 20-21 correspond to *kcnma1* DNA reverse transcribed from the corresponding RNA and partially from plasmid DNA.

Figure S1.2 Increasing the amount of cDNA to 10µl permits detection of *slo-1* in wild-type non-transformed worms.



15µl of DNA was separated on 1.5% Agarose gel next to 100bp DNA ladder (GeneStar). Lane 1: *slo-1* DNA reverse transcribed from 4µg of total RNA extracted from *N2 C. elegans*. 10µl cDNA was used for PCR (35 cycles) in the second step of RT PCR. Lane 2: control for the reverse transcription of *slo-1* RNA extracted from *N2* (no reverse transcriptase). Lane 3: *slo-1* DNA reverse transcribed from 4µg of total RNA extracted from *slo-1(js379) C. elegans*. 10µl cDNA was used for PCR (35 cycles) in the second step of RT PCR. Lane 4: control for the reverse transcription of *slo-1* RNA extracted from *slo-1(js379)* (no reverse transcriptase). Lane 5: 10ng of pBK3.1 (positive control for the *slo-1* amplification). Lane 6: negative control for the *slo-1* amplification (DNA was substituted with dH_2O). Bands of 425 base pairs in the lanes 1 and 3 correspond to *slo-1* DNA reverse transcribed from *slo-1* RNA extracted from *N2* and *slo-1(js379) C. elegans* respectively. It was not possible to detect reverse transcribed *slo-1* extracted from *N2* and *slo-1(js379) C. elegans* when 2µl of cDNA was amplified (Figure S1.1). Increasing cDNA 5 fold enabled detection of *slo-1* in these worms. Thus, native expression of *slo-1* in wild-type *N2* is at least 5 times lower than in transgenic worms. A band in lane 3 is much fainter than the band in lane 1 suggesting that *slo-1* mRNA is present in *slo-1(js379)* mutant at very low amounts and/or is unstable.