Exploring the Developmental Effects of Antidepressants in *Caenorhabditis elegans*

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Antidepressants

Background

Selective Serotonin Reuptake Inhibitors (SSRIs) and Selective Norepinephrine Reuptake Inhibitors (SNRIs) are the most common antidepressant prescribed to treat major depressive disorder.

Side effect issues

As is with most drugs, antidepressants do not exist currently without potentially harmful side effects.

- These associated side effects may be correlated with the toxicity of medications.

Importance

Using animal models could help to screen for these potential toxic side effects before testing in humans is carried out, reducing harm and increasing benefits.

TABLE 2

Adverse effects of antidepressant drugs, based on mechanism of action

Norepinephrine transporter blockade
Anxiety
Augmentation of pressor effects of sympathomimetic amines
Diaphoresis
Tachycardia
Tremor

Serotonin reuptake inhibition
Anorexia early in the treatment and weight gain later
Dose-dependent increase or decrease in anxiety
Ejaculatory disturbances, anorgasmia, and decreased libido
Extrapyramidal side effects
Interaction with monoamine oxidase inhibitors and tryptophan
Nausea, vomiting, and diarrhea.
Sedation or insomnia
Serotonin syndrome

Dopamine reuptake inhibition
Activation and aggravation of psychosis
Parkinsonism
Psychomotor activation

Khawam EA, Laurencic G, Malone DA Jr. Side effects of antidepressants: an overview. Cleve Clin J Med. 2006 Apr;73(4):351-3, 356-61. doi: 10.3949/ccjm.73.4.351. PMID: 16610395.

Pharmaceutical Trials and Animal Toxicity

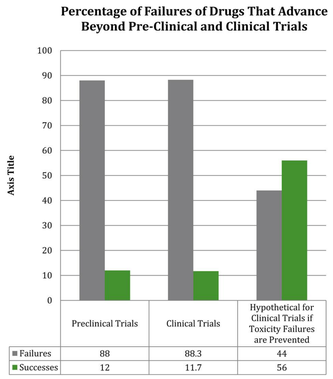
Animal testing has been widely used to predict efficacy and safety of pharmaceuticals in humans.

Reasons for Animal Testing

- Animal toxicity testing required by FDA since 1938
- Mice and rats share 85% analogous DNA with humans
- Animals involved in the development of vaccines, pharmaceuticals, and medical procedures

Downsides of Animal Testing

- Costs $2-4 million per trial on average
- 88% of Preclinical trials fail
- Questionable reproducibility, reliability



Van Norman GA. Limitations of Animal Studies for Predicting Toxicity in Clinical Trials: Is it Time to Rethink Our Current Approach? *JACC Basic Transl Sci*. 2019;4(7):845-854. Published 2019 Nov 25. doi:10.1016/j.jacbts.2019.10.008

Caenorhabditis (C.) elegans

C. elegans are transparent nematodes that are about ~1 mm in length when fully matured.

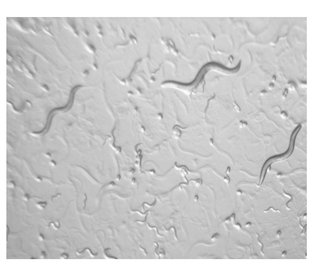
C. elegans have a rapid, observable life cycle. They are also easy to cultivate, relatively inexpensive, and non-hazardous to humans.

Pros of *C. elegans* as a Model

- 83% of proteins in *C. elegans* have homologous proteins in humans. Overall, 50% genetic similarity to humans. Serotonin transporter present as seen in humans.

Cons of *C. elegans* as a Model

- Not a whole organ species, difficult to directly translate human pathology into *C. elegans* phenotypes



Eggs, larvae, and adults of *C. elegans*

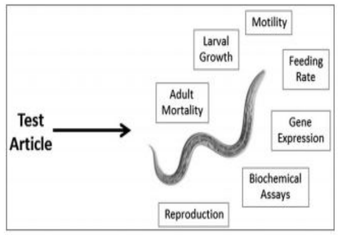
Objective and Strategies

Objective

- To determine if various antidepressants have toxic side effects on *C. elegans* through observing their development and egg laying behavior after exposure.

Strategies

- Dissolve SSRIs and SNRIs in different solvents (H_2O or ethanol)
- Expose *C. elegans* to SSRIs and SNRIs and examine if there is:
  - an increase or decrease in overall size of the nematode.
  - an increase or decrease in the total number of eggs laid per adult nematode.



Hunt et al, 2016 Journal of Applied Toxicology

Methods and Materials

Strain of worm used: N2
Fed with: *E. coli* based OP50

Day 0

- Drug plates are prepared by pipetting dilute drug solution onto small agar plates
- Worms are bleached to synchronize the population
- Bleached worms (egg-L1) are then pipetted onto the prepared drug plate

Day 1

- Seed the previously prepared drug plate with OP50
- Worms are in the L2-L3 stage

Day 2

- Four nematodes are picked from the drug plates onto regular sized plates for the later egg counts
- The remaining worms on the drug plates are anesthetized with sodium azide and measured with an eyepiece reticle on a compound microscope
- Worms are in the L4/young adult stage

Day 3

- Egg count day one. Larvae, egg, and adult nematodes are counted.

Day 4

- Egg count day two. Larvae, egg, and adult nematodes are counted.

Egg Laying and Sizing- Desipramine

A B

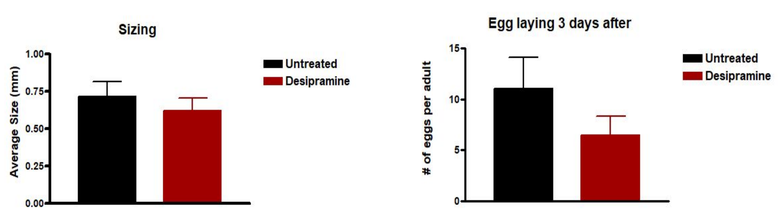


Figure 1 - Toxicity of desipramine , a tricyclic antidepressant, dissolved in water. (A) Size of desipramine treated worms vs untreated (n=7). (B) Number of eggs laid (eggs + larvae) per adult after 3 days (n=7).

Egg Laying and Sizing - SNRIs (Levomilnacipran and Milnacipran)

A B

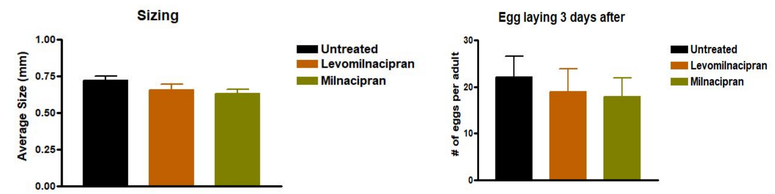


Figure 2 - Toxicity of selective norepinephrine reuptake inhibitors dissolved in water. (A) Size of SNRI- treated worms vs untreated (n=10). (B) Number of eggs laid (eggs + larvae) per adult after 3 days (n=13).

Egg Laying and Sizing- SSRIs (Paroxetine, Citalopram, Escitalopram)

A B

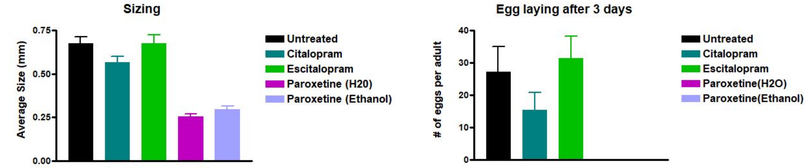


Figure 3 - Toxicity of selective serotonin reuptake inhibitors dissolved in water, and ethanol (paroxetine) (A) Size of SSRI- treated worms vs untreated (n=5-10). (B) Number of eggs laid (eggs + larvae) per adult after 3 days (n=5-10).

Conclusions and Future Directions

Conclusions

Our results indicate that:

- Desipramine showed little to no toxic effects in development, however some significance was shown when comparing the egg laying to an untreated population of nematodes.
- There was no significant difference in the size of the SNRI treated nematodes compared to untreated.
- Of the SSRIs tested, paroxetine treated worms had the most toxic developmental effects, while escitalopram treated worms exhibited the least toxic effects.

Future Directions

- Would be interesting to look at other SSRIs to see if any have a similar toxic effect as paroxetine.
- Also, would be interesting to try different types of testing on the *C. elegans*, such as swim testing.

Disclosure

All authors of this presentation have nothing to disclose concerning possible financial or personal relationships with commercial entities that may have a direct or indirect interest in the subject matter of this presentation.