Evaluating the therapeutic effects of midazolam on planaria exposed to convulsant chemicals

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Introduction

Planaria (*Dugesia dorotocephala*) are a species of the flatworm organism group which are often aquatic. Planaria demonstrate planarian seizure-like activity (pSLA) in response to chemoconvulsants. During pSLAs, the planaria began to convulse in and out of a C-shape (C-shape hyperkinesias) or make a corkscrew motion with their bodies. pSLAs usually last for a few minutes at a time. Previous research has shown planaria to be viable organisms for neurotoxicology studies. A neurotoxicology study has shown that planaria have reacted to cocaine only when they are not in the regenerating phase (Baker et al., 2013). This study validated planarian use by indicating planarian reactions are due to a complex nervous system and not automatic muscular response. The convulsant chemicals used in this study were picrotoxin (PTX), a toxic plant derivative, and the nerve agent soman (GD). Both PTX and soman have been studied and shown to cause a significant amount of pSLAs. Therefore, this study assesses whether the treatment drug of midazolam can inhibit the pSLAs for an extended time.

Midazolam (MDZ) is a drug that is effective in humans for treating generalized seizures, status epilepticus, and behavioral emergencies where sedation is required for the safety of the patient (Nordt & Clark, 1997). Midazolam works faster and is safer than many similar drugs (Nordt & Clark, 1997). These qualities made it a feasible drug to be researched for treatment of chemical-induced seizures.

The purpose of this study was to quantify and evaluate the therapeutic effects of midazolam on planaria exposed to convulsant chemicals

Materials and Methods

Each convulsant chemical was tested with separate concentration ranges based on its potency. PTX was tested alone from the 10–2,000 micromolar (μM) concentrations, 2,000 μM caused the most pSLAs. GD was tested at both the 1 and 5 μM level with midazolam treatment (GD exposure performed by MRICD lab technicians; GD previously tested alone). Midazolam was tested from10–40 μM range for GD and PTX. Each planaria was placed in its own well of a 24-well plate with 1000 microliters (μL) of the chemical +/− midazolam solution.

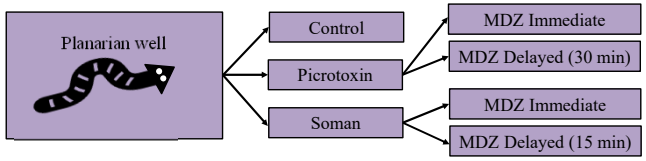


Figure 2 (above): Experimental procedure of planaria exposure to neurotoxins and treatment.

Materials and Methods (continued)

There were a total of six trials performed for each experimental group. Each trial ran for one hour. The concentrations of convulsant chemicals and treatment were distributed by column in the well-plates (Figure 1). For the trials evaluating midazolam treatment there was an immediate experimental group in which the planaria were exposed to the convulsant chemical and midazolam simultaneously. Experimental groups were tested on two different time frames shown in Figure 2.

EthoVision XT, a software used to track behavior and movement of, activity of a planaria is defined as the mean total movement throughout the well over one minute. Data points were recorded each minute during the 60-minute trial. Manual review of videos recorded for each trial confirmed that spikes in activity measurements correlate with observed pSLAs. Activity values at each time point were averaged across the six trials by four wells per concentration ($n = 24$), and these values were used in statistical testing.

Results

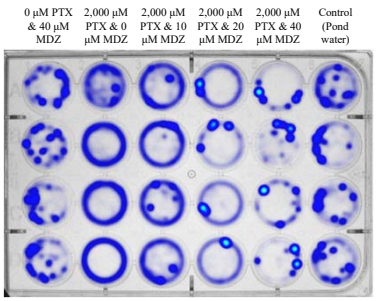


Figure 3 (left): Heatmap of planarian activity from a trial testing immediate midazolam treatment. This heatmap follows the concentration distribution shown at the top of each column. Each well holds up to 57 mL and is 1.6 cm in diameter. The lid was removed prior to trials. Quality of image reduced based on limitations of imaging hardware.

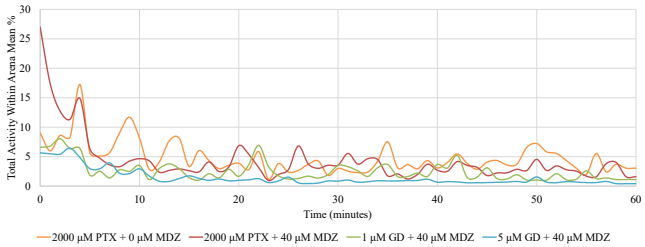
Two-way ANOVAs were used to evaluate the effect of time and midazolam concentration on the activity values of the planaria. For 2,000 μM PTX exposure with midazolam treatment, there is a significant effect of both time of treatment (immediate versus delayed) and concentration of treatment with $p < .001$. For the GD exposure at 1 μM and 5 μM, there was a significant effect of both time of treatment (immediate versus delayed) and concentration of treatment with $p < .001$.

Table 1 (right): Descriptive statistics for activity values produced by three separate two-way ANOVAs. Higher midazolam concentration correlated to a lower mean in activity values of each test. PTX at 2,000 μM/0 μM midazolam had a decrease in activity due to worm death over trial.

Concentration of Convulsant (μM)	Concentration of Midazolam (μM)	Mean	Standard Deviation
PTX 2000	0	4.814	2.701
	10	5.767	2.919
	20	5.024	3.881
	40	4.376	4.232
GD 1	10	2.623	1.711
	20	2.083	1.077
	40	1.822	0.896
GD 5	10	1.477	1.432
	20	1.194	1.239
	40	1.077	0.843

Results (continued)

Planaria exposure picrotoxin and midazolam immediate treatment



Graph 1 (below): Results from PTX and midazolam (MDZ) immediate treatment activity values averaged. Spikes in graph indicate pSLAs. 40 μM concentration of midazolam for both PTX and GD was the most effective ($p < .001$). 5 μM of GD caused death in planaria after 20 minutes regardless of treatment, as indicated by low total activity.

Conclusions

The purpose of this study was to evaluate the therapeutic effects of midazolam in planaria exposed to PTX and GD by utilizing the activity measured by the movement-tracking software, EthoVision XT. The results showed that the highest concentrations of midazolam (40 μM) have a statistically significant effect on activity values for both PTX and GD. Midazolam inhibited activity in planaria exposed to PTX on the immediate and delayed scales. However, during the experiments it was observed that those treated with midazolam still had pSLAs (spikes in activity) after 20 minutes post-treatment (Graph 1). GD exposure with immediate and delayed midazolam treatment showed inhibition of seizures, once again only for the first 20 minutes during the trial time. In conclusion, the results indicate that midazolam can temporarily inhibit pSLAs in planaria exposed to convulsant chemicals. Future research should include more advanced movement-tracking analyses to better quantify pSLAs. In addition to this, a higher concentration of midazolam could be tested to see if it can inhibit pSLAs for an extended time frame.

References

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Nordt S. P., & Clark R. F. (1997). Midazolam: A review of therapeutic uses and toxicity. *The Journal of Emergency Medicine, 15*(3), 357–365. doi: 10.1016/S0736–4679(97)00022–X