Analyzing neuronal necrosis to Sprague-Dawley rats exposed to chemicals of interest

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Introduction

Chemical weapons like nerve agents and toxic industrial chemicals have been used by governments and terrorists to perpetrate fatal attacks (McLeod, 1985). Chemicals that cause brain damage are particularly concerning because they have lasting effects on the quality of survivors' lives. The human brain has many regions that are important to the functionality of the body. For example, the amygdala is responsible for controlling emotions. Another critical region is the cerebral cortex that has numerous functions such as motor function, visual comprehension, and processing sounds (Molenberghs et al., 2012). In addition to functions specific to certain brain regions, these regions connect to create networks that rely heavily on each other to perform (Garman, 2011). Damage to one brain region can affect many different neurological processes.

The connections made in the brain are all performed by neurons which are cells that vary in size to transmit information within the brain. A measure of healthiness within the brain is often through levels of neuronal necrosis or cell death in the brain. Necrotic neurons appear shrunken, heterogenous to each other, stained red, less Nissl substance, and have a fragmented or dissolved nucleus (Garman, 2011).

The purpose of this study was to determine which chemicals of interest (COI) would produce neuronal necrosis and the extent of neuron degeneration in select regions of the brain.

Materials and Methods

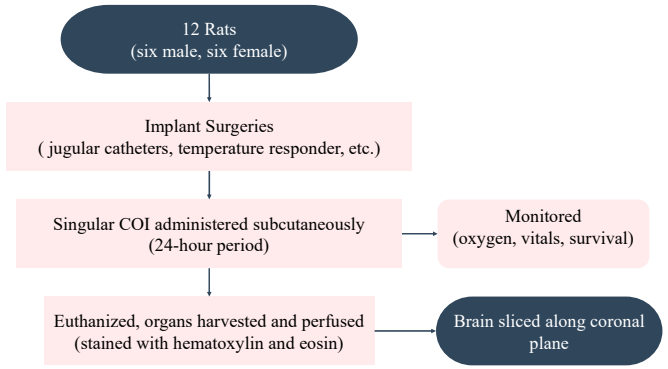

Figure 1 (above): The flowchart describes the process of COI administration to the rats within the set. Information including the COI tested, measurements and supportive measures (oxygen and fluids) taken in the study are blinded to the pathologists. The four bregma locations in slicing include 1.00 mm, −2.92 mm, −6.36 mm, and −9.00 mm.

Materials and Methods (continued)

Following the procedure displayed in Figure 1, the pathologist receives four slides per rat, for all rats. The four slides per rat were reviewed for neuronal necrosis in which the number of rats per chemical with necrosis were recorded. When a rat was found to have necrosis, the individual regions across all four bregma cross sections were scored on the severity of necrosis. The scale used to rate the severity of necrosis is a zero to four scale. A severity rating of zero is less than 1% necrosis, a score of one is 1–10%, two is 11–25%, three is 26–44%, and four is greater than 45%. These ratings are applied to the following regions: hippocampus, amygdala, thalamus, cerebral cortex, piriform cortex, and the caudate putamen. Each region is scored independently. Once all slides are rated, the pathologists are unblinded to the chemical identities, for connections and conclusions to be made from the severity ratings. This process is repeated for all 10 COIs listed in Table 1 with varied dosages to induce detectable observations.

Results

The findings of this study produced numerous images of necrosis within several brain regions across a multitude of the chemicals of interest as seen in Figure 2. As shown in Table 1, chemicals captamine, fluoroacetamide, hydrazine, 2-fluoroethanol, methyl hydrazine and hydroxyacetonitrile produced rats which displayed behavioral signs of central nervous system damage (CNS) such as seizures or convulsions.

Chemical	Rats Tested	Survival	CNS Displayed	Necrosis Present
Mecuric Chloride	12	8	0	0
Ehtylenediamine	12	12	0	0
N-ethylmaleimide	12	12	0	0
2,4-dinitrophenol	12	11	0	0
Captamine	12	9	3	0
2-Fluoroethanol	12	0	12	0
Fluoroacetamide	12	4	10	0
Hydrazine	12	6	12	0
Methyl Hydrazine	12	1	12	1
Hydroxyacetonitrile	12	3	4	3

Table 1 (above): The table is representative of the survival, CNS behavior exhibited, and pathology for each set of rats (n = 12). The necrosis present was observed for 12 rats within each COI, however, methyl hydrazine and hydroxyacetonitrile had fewer rats for review due to the lethality of the chemicals. The rats reviewed were one rat and three rats, respectively.

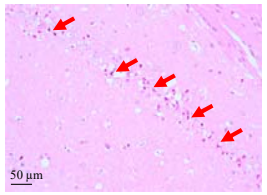

Figure 2 (right): Brain, hippocampus, methyl hydrazine group. Neuronal necrosis (red arrow). Necrotic neurons are shrunken, have deeply eosinophilic (red) cytoplasm, and have a small, condensed or fragmented nucleus. Hematoxylin and eosin (H&E) stain with 20× magnification.

Results (continued)

Despite six chemicals displaying behavioral signs of CNS, only two of the chemicals, methyl hydrazine and hydroxyacetonitrile presented necrotic pathology within any of the brain regions. Furthermore, certain chemicals did exhibit higher severity ratings in specific regions of the brain. In the hydroxyacetonitrile group, three of the surviving rats resulted in the caudate putamen receiving the highest rating ranging from a severity of one to four as expressed in Figure 3. The results of this study produced beneficial and significant findings.

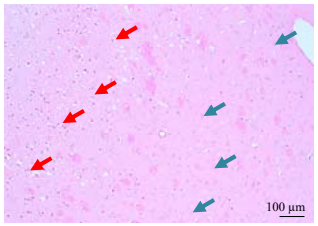

Figure 3 (left): Brain, caudate putamen, hydroxyacetonitrile group. Neuronal necrosis (red arrow) affecting the caudate putamen and adjacent normal cerebral cortical neurons (blue arrows). The location of necrosis in this rat is consistent with the other two rats exposed to hydroxyacetonitrile. Hematoxylin and eosin, 10× magnification.

Discussion

The purpose of this study was to determine if COIs cause neuronal necrosis and, if so, the location and extent of the damage. The findings concluded that chemicals methyl-hydrazine and hydroxyacetonitrile caused neuronal necrosis. Within these chemicals, a multitude of regions were affected namely the amygdala, hippocampus, cerebral cortex, and caudate putamen at varying degrees. Although various chemicals caused behavioral indications of CNS damage, but did not have pathology, it is hypothesized that the rats did not survive long enough for histologic evidence of necrosis to develop.

The results of this study are preliminary findings. The chemicals that did not cause neuronal necrosis should continue to be studied for other physiological effects to the body as the chemicals could still have toxic effects on other organ systems. Future experiments will focus on preventing and treating brain damage caused by COIs.

References

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