May 9, 2011

Ms Catherine Langman
Co-ordinator
Coronial Services Unit
Ministry of Justice

Re: Histological assessment of CNS samples

Dear Ms Langman:

The following report summarizes our findings following a detailed histological assessment of the CNS samples from Jasmine Renata provided by the coroner's office.

Methods: Histological preparation and stains used

The material used consisted of three blocks of CNS tissue prepared by the coroner's office. These included: cerebellum, hippocampus, and watershed cortex. Regions that might have been more relevant given the symptoms prior to death, e.g., brain stem and cervical cord, were not provided. Sections were cut at 5 µm on a Leica cryostat and mounted on glass slides. The following stains were employed following standard protocols.

Iba1 - for activated and resting microglia [1-3]
GFAP - for reactive astrocytes [1, 3-6]
Hsp70 – for heat shock protein 70, stress marker [5, 6]
ATF1 – for activating transcription factor 1, stress marker [7]
Casp-3 - for apoptotic (programmed) cell death [1, 3]
FloroJade B - for necrotic cell death [1]
Tau - for hyperphosphorylated Tau [3]
Modified hematoxylin - for aluminum [8]

Stained sections were examined using a Zeiss Axiovert (Carl Zeiss Canada Ltd., Toronto, ON) microscope zoom at x20 and x40. Images were captured using AxioVision 4.3 software. As the sections represented material from only one individual, no statistical evaluation was possible, although we have tried to scale the intensity of staining and the subgroups of cells stained using a qualitative measure (see Tables 1 and 2, Appendix).

Results

Apoptosis: Apoptotic cells labelled with caspase 3 (Casp-3) were seen in hippocampus and cerebellum (Figs. 1&2, Appendix). In the hippocampus, Casp-3 stained a large number of pyramidal neurons but labelling was weak (Fig. 1A). Note, however, that a sub-populations of neurons in the hippocampus showed intense punctate staining with the Casp-3 (Fig. 1B) and also with modified hematoxylin (Fig. 1C-D).
Cerebellar Purkinje cells showed Casp-3 positive staining apparently around the nucleus (Fig. 2A-B). Modified hematoxylin stained Purkinje cells in a dense punctate pattern (Fig. 2C-D). Watershed cortex did not show Casp-3 labelling (Fig. 3A).

Neuroinflammation markers:
Microglia:
Iba-1 showed dense labelling of activated microglia in the hippocampus (Fig. 4A-B). Some of these stained cells appeared to be in the transitionally activated form seen following various neuronal insults (Table 3). The labelled cells are more highly branched with thicker and more numerous processes and a more elongated cell body (Fig. 4A-B). Note that in the cerebellum (Fig. 4C-D) and the watershed cortex (Fig. 4E-F), the stained microglia exhibit a ramified morphology, which is typical for resting microglia [1, 2, 9] with mainly round cell bodies and long very thin processes (Fig. 4C-F).

Astrocytes:
GFAP showed a high number of reactive astrocytes in the cerebellum (Fig. 5) and watershed cortex (Fig. 6A-B) but none in the hippocampus (Fig. 6C).

Stress markers:
In the cerebellum, a high proportion of Purkinje cells showed intense staining with ATF-1 (Fig. 7A-C) and Hsp70 (Fig. 7D-F) throughout the cell bodies. In the hippocampus, ATF-1 showed very strong staining in a high proportion of pyramidal neurons (Fig. 8A-C). Similar pattern was observed for Hsp70 (Fig. 8D-F) albeit the staining was of slightly lesser intensity.
In the watershed cortex, only ATF-1 showed intense staining of neuronal cells (Fig. 9A-B); Hsp70 stained neurons more weakly (Fig. 9C-D).

Presence of aluminium:
Aluminum labelling was present in a punctuate pattern of staining in all areas examined with the hippocampus (Fig. 1D-F) and cerebellum (Fig. 2D-F) having the highest apparent numbers of labelled neurons.

Other markers (Tau and FluroJade B) for hyperphosphorylated tau and cellular necrosis:
No sections examined for the three areas showed tau or FluroJade B labelling.

Conclusions:
The cellular stains described above show some apparent abnormalities. These include:
1. A number of apoptotic cells in hippocampus and cerebellum;
2. Activated microglia particularly in the hippocampus;
3. The presence of stress markers in cerebellum and hippocampus;
4. The presence of aluminium labelling in all areas tested.

Caveats:
The obvious caveats to the present results are that the samples represents one individual against which there are no controls. For this reason, our conclusions are necessarily limited in scope. However, apoptotic cell death in at least two regions of CNS accompanied by activated glial and cellular stress markers in these same areas suggests an underlying pathological process involving the CNS. Aluminum labelling in all areas tested could be involved in this process, although the source of the aluminium cannot be determined from the current assays.

According to the European Medicines Agency (EMEA) Pathologists Panel for evaluation of sudden unexplained death as a possible sequelae to vaccination, the identification of a possible pathological basis of reflexogenic mechanisms necessarily requires examination of the brainstem nuclei and of the cardiac conduction system on serial sections [10]. The brainstem plays an important role in the regulation the function of the cardiac and respiratory systems as well as the central nervous system. It is also crucial in maintaining consciousness and regulating the sleep cycle. For this reason, the brainstem would be an obvious choice for pathological examination in cases of vaccine-suspected unexplained deaths. In regard to cases where a particular vaccine is suspected (e.g., Gardasil), the rationale for brainstem examination is further supported.

Thank you for the opportunity to examine this material.

Sincerely yours,

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