Clinical and Pathological Aspects and Cerebellar Lectin Binding in Cattle Poisoned With *Solanum fastigiatum* var. *fastigiatum* and *Solanum bonariense*

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Abstract

Microscopic and lectin histochemical studies were performed using the cerebella of 33 natural cases of *Solanum fastigiatum* var. *fastigiatum* intoxication in cattle from southern Brazil and 2 natural and 4 experimental cases of *Solanum bonariense* from Uruguay. The following biotinylated lectins were used in both cases: WGA, sWGA, BS-I, Con-A, RCA-I, DBA, and UEA-I, with the addition of LCA in *S. fastigiatum* poisoning cases. Histologically, the lesions consisted of fine vacuolization, distention of portions of the Purkinje cells, axonal spheroids measuring 14-50 µm in the granular cell layer and adjacent white matter and, proliferation of the Bergmann's glia. Lectin histochemistry revealed strong reactivity of stored material in Purkinje neurons with the lectins sWGA, Con-A, and LCA in *S. fastigiatum* cases. A similar pattern was found in *S. bonariense* cases with a most intense reaction to WGA, and less intense reaction to Con-A, whereas BS-I and RCA-I binding was absent to poor in these neurons in all the cases studied. Lectin reactivity in Purkinje cells between cases was independent of cell damage (from mild to severe loss of neurons). Both *S. fastigiatum* and *S. bonariense* have similar lectin binding, suggesting a similar pathogenesis. Since comparable binding patterns have been described in animals poisoned with swainsonine-containing plants, perhaps the toxins in these plants contain related glycosidase-inhibiting toxins or inhibit glycoprotein and lysosomal metabolism through some related mechanism. The results of this study showed that in spontaneous poisoning by *S. fastigiatum* and *S. bonariense* in cattle, the pattern of lectin binding is similar to those observed in *S. fastigiatum* experimental conditions.

Keywords: Cattle, cerebellar degeneration, lectin histochemistry, neurotoxicity, poisoning, *Solanum fastigiatum, S. bonariense*

Introduction

Solanum fastigiatum var. *fastigiatum* and *S. bonariense* intoxication is an important cerebellar disorder in cattle in southern Brazil and Uruguay (Riet-Correa et al. 1983, Rech et al. 2006, Verdes et al. 2006). *S. fastigiatum* is one of the major plant poisonings in cattle in Rio Grande do Sul, a state in

southern Brazil (Rissi et al. 2007). Similar disorders induced by other species of *Solanum* have been reported in cattle grazing *S. dimidiatum* and in goats grazing *S. viarum* in the United States (Menzies et al. 1979, Porter et al. 2003) and cattle eating *S. kwebense* (*S. tettense*) in South Africa (Pienaar et al. 1976, van der Lugt et al. 2010) and *S. cinereum* in Australia (Bourke 1997). *S. fastigiatum* poisoning was reproduced in sheep that developed similar lesions to those observed in cattle (Zambrano et al. 1985). Poisoning by *S. paniculatum* in cattle in northeastern Brazil exhibited similar clinical signs and pathological lesions as those caused by *S. fastigiatum* (Medeiros et al. 2004).

The neurological disease of *S. fastigiatum* and *S. bonariense* poisoning is characterized by periodic episodes of seizures, loss of balance, nystagmus, opisthotonus, tremors, hypermetria, extension of the neck, head tilt, and ataxia (Riet-Correa et al. 2009). The age of affected cattle ranges from 6 months to 10 years. In some animals a loss in body condition is observed. Death is uncommon and some animals have to be euthanized because of severe injuries caused by repeated falls.

Grossly, the brain appears normal or cerebellar atrophy is observed (Rech et al. 2006). Histologic lesions include fine vacuolization and diffuse loss of Purkinje cells, axonal spheroids in the granular layer and white matter, Bergman gliosis in the molecular layer, and atrophy of molecular and granular layers. Ultrastructurally, there are numerous lipid inclusions and membranous bodies in the cytoplasm of Purkinje cells (Riet-Correa et al. 1983, Barros et al. 1987). A lectin histochemical study of the cerebella of two cattle experimentally poisoned with *S. fastigiatum* showed accumulation of specific oligosaccharides and other terminal sugars in the degenerated cells, suggesting a glycolipid storage disease (Paulovich et al. 2002). However, the lectin-binding patterns in cerebella of cattle naturally poisoned with *S. fastigiatum* var. *fastigiatum* or naturally and experimentally poisoned with *S. bonariense* have not been documented. Lectin histochemistry using paraffin-embedded sections may be useful in the identification of specific sugars and hence aid in diagnosing glycoprotein and glycolipid storage diseases (Alroy et al. 1984, Alroy et al. 1986, Driemeier et al. 2000, Paulovich et al. 2002). The objectives of this retrospective study were to describe the clinical signs and compare the morphological and lectin histochemical findings of 33 natural cases of *S. fastigiatum* var. *fastigiatum* intoxication in cattle from southern Brazil and 2 natural and 4 experimental cases of *S. bonariense* poisoning from Uruguay.

Material and Methods

Thirty-three cases of spontaneous *S. fastigiatum* var. *fastigiatum* poisoning in cattle occurring from 2006 to 2009 were evaluated. The cases originated from three municipalities of central Rio Grande do Sul State, southern Brazil. Additionally, two heifers naturally poisoned with *S. bonariense* on farms in Soriano, western Uruguay, and four cattle experimentally intoxicated with the same plant and one control were used. Details of the experimental study were previously reported (Verdes et al. 2006).

The history and clinical signs were obtained from the owners or the practicing veterinarians in all cases. At necropsy, the brains were collected and fixed in 10% buffered neutral formalin for 5 to 7 days. The following sections of the brain were evaluated histologically: medulla at the level of the obex, pons, cerebellum, midbrain at the level of the rostral colliculus, thalamus, basal nuclei, hippocampus, and frontal, parietal, and occipital lobes. The samples were routinely processed and stained with hematoxylin and eosin (HE).

For the lectin histochemical study, after deparaffination with xylene, additional samples of cerebellum were immersed in 0.3% hydrogen

Table 1. Lectins used in the histochemical study and their major specificities

Acronym	Source	Major specificity[a]
WGA	*Triticum vulgaris,* Wheatgerm	D-N-acetyl chitobiose, N-acetyl lactosamine and some sialyl residues
sWGA	Succinyl-WGA	β-(1-4)-D-N-acetyl-glucosamine
BS-I	*Bandeirea simplicifolia*-I	α-D-Galactose
Con-A	*Concanavalina ensiformis*	α-D-glucose and α-D-mannose
RCA-I	*Ricinus communis*	β-D-galactose
DBA	*Dolichos biflorus*	α-D-N-acetyl-galactosamine
UEA-I	*Ulex europaeus*-I, Gorse	α1,2-linked fucosyl residues
LCA	*Lens culinaris*	D-Mannose and D-Glucose

[a]Goldstein and Hayes (1978)

peroxide in methanol for 30 min at room temperature, rinsed several times in 0.01 M phosphate-buffered saline (PBS), pH 7.2, and submerged in PBS containing 0.1% bovine serum albumin for 15 min. They were then incubated with the eight biotinylated lectins (Vector Laboratories Inc., Burlingame, CA, USA) shown in Table 1. The optimal concentration for each lectin, which allowed maximum staining with minimum background, was at a dilution of 30 µg/mL in PBS for 1 h followed by incubation with avidin-biotin-peroxidase complex (ABC) (Vector Laboratories Inc.) for 45 min. The horseradish peroxidase was activated by incubation for 4 to 10 min with buffered0.05 M Tris-HCl solution, pH 7.6, containing 0.02% diaminobenzidine (DAB) and 0.05% H_2O_2.

All sections were counterstained with Mayer's hematoxylin. The following negative controls were performed: the lectins were omitted or blocked by incubating them with their blocking sugars (0.1-0.2 M in PBS) for 1 h at room temperature before application to the sections. In the protocol for *S.fastigiatum*, cerebellum of one 3-year-old bovine without neurological signs and morphological lesions in the brain was used as a control. In the *S. bonariense* experiment, unaffected cerebellar samples were obtained from a 1-year-old bovine on the same farm which was also used as a control during the experimental reproduction (Verdes et al. 2006). The lectin binding was analyzed using the following semiquantitative scale of stained structures and subjectively scored as follows: (0) none, (1) weakly positive, (2) moderately positive, and (3) strongly positive. Two pathologists, blinded to previous procedures, evaluated the sections.

Results

Neurological clinical signs observed in all affected cattle included proprioceptive and cerebellar deficits such as incoordination, hypermetria, tremors, frequent falls, and transitory seizures when moved or stimulated. Seizures were sporadic and limited to a few seconds at a time. After these episodes, numerous animals assumed a wide base stance or appeared apparently normal. Progressive weight loss was observed in many animals.

Gross change was noted only in two cattle naturally poisoned with *S. fastigiatum* that included moderate atrophy of the cerebellum. Microscopically, the lesions were restricted to the cerebellum and consisted of fine vacuolization and distention of portions of the Purkinje cells; some degenerated cells had swollen and eosinophilic cytoplasm and others had peripherally placed nuclei. In some cases, the vacuoles were confluent and occupied one pole or the most of the pericarya of the neurons. Axonal spheroids measuring 14-50 µm were visualized frequently in the granular layer and adjacent white matter. In chronic cases, there were severe loss of Purkinje cells in many folia and proliferation of the Bergmann's glia. Occasionally, focal gliosis and mild histiocytic perivascular cuffs were visualized in the white matter of the cerebellum of three cases. The control animals did not have cerebellar lesions. No alterations were observed in other organs.

There were different lectin binding patterns between the affected and control cerebella. Results are summarized in tables 2 and 3. In the *S. fastigiatum* study, there was a clear binding affinity of the cytoplasmic vacuoles in the Purkinje cells for sWGA (figure 1), LCA (figure 2), and Con-A (figure 3). Similar results were achieved for sWGA in natural and experimental *S. bonariense* cases and notable, although less intense reaction, was observed for Con-A. Histochemical marcation was also different for WGA lectin with a moderate reaction in *S. fastigiatum* cases (figure 4) and more accentuated in the *S. bonariense* cerebellum; BS-I and RCA-I binding was absent to poor in all the cases studied. DBA and UEA-I binding was absent in all the cases. Lectin reactivity in Purkinje cells between cases was independent of cell damage stage (from mild to severe loss of neurons). Furthermore, BS-I lectin presented marked binding in the endothelium of blood vessels in all affected and normal cerebella. Purkinje cells of the control bovines showed null to weak reactivity for sWGA, WGA, and Con-A.

Discussion

The clinical signs and gross and microscopic findings observed in cattle in the current study are similar to those previously described in *S. fastigiatum* and *S. bonariense* poisoning in cattle (Riet-Correa et al. 1983, Zambrano et al. 1985, Rech et al. 2006, Verdes et al. 2006). A fine vacuolar degeneration of neuronal perikaria with progressive axonal degeneration resulting in the death of these cells and eventual atrophy of the cerebellum are the main pathological features of this disease. Other species such as *S. dimidiatum*, *S. kwebense* (*tettense*), *S. viarum*, and *S. cinereum* have been associated with neurological disorders in ruminants

Table 2. Intensity of lectin binding in Purkinje cells of *S. fastigiatum* var. *fastigiatum*-poisoned and control cattle

WGA	sWGA	BS-I	Con-A	RCA-I	DBA	UEA-I	LCA
0-2[a] (0-1)	2-3 (1)	0-1 (0)	3 (0-1)	0-1(0)	0 (0)	0 (0)	2-3 (0)

[a]Numbers indicate intensity of binding on a subjective scale of 0 (no reactive) up to 3 (maximum reactivity). Control results from normal cattle are provided in the parentheses.

Table 3. Intensity of lectin binding in Purkinje cells of spontaneous and experimental *S. bonariense*-poisoned and control cattle

	WGA	sWGA	BS-I	Con-A	RCA-I	DBA	UEA-I
Spontaneous	2-3[a] (1)	2 (0-1)	0 (0)	2 (0-1)	0 (0)	0-1 (0-1)	0-1 (0-1)
Experimental	2 (1)	2-3 (0-1)	0 (0)	1-2 (0-1)	0-1(0)	0-1 (0-1)	0-1 (0-1)

[a]Numbers indicate intensity of binding on a subjective scale of 0 (no reactive) up to 3 (maximum reactivity). Control results from normal cattle are provided in the parentheses.

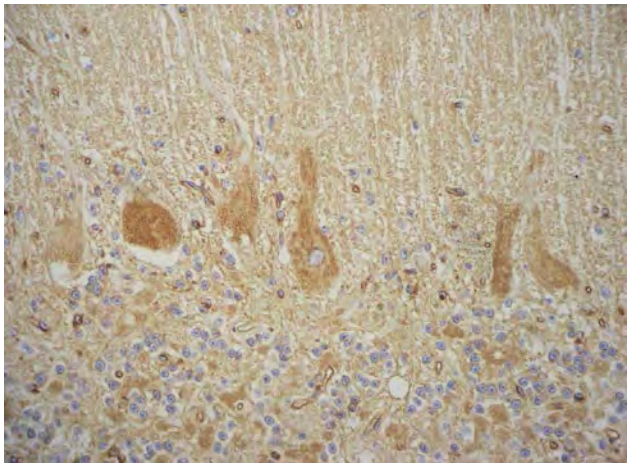

Figure 1. Cerebellum from a bovine naturally poisoned by *Solanum fastigiatum*. Strong binding to sWGA in the cytoplasm of Purkinje cells. LHQ, Mayer's hematoxylin counterstain, 40X.

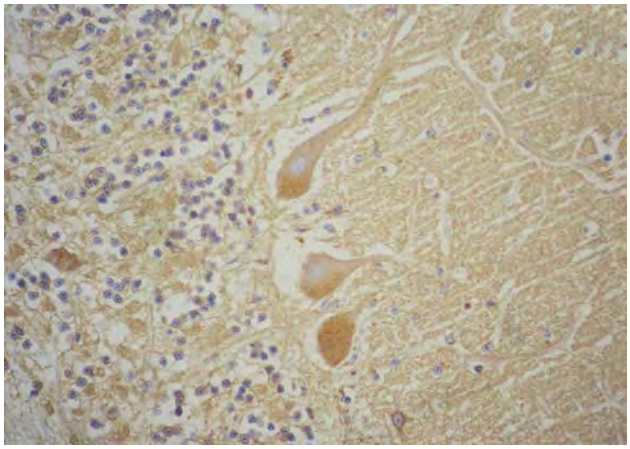

Figure 2. Cerebellum from a bovine naturally poisoned by *Solanum fastigiatum*. Moderate to strong binding to LCA in the cytoplasm of Purkinje cells. LHQ, Mayer's hematoxylin counterstain, 40X.

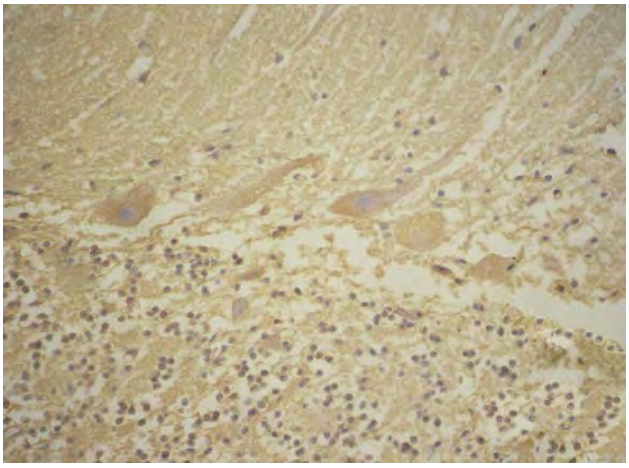

Figure 3. Cerebellum from a bovine naturally poisoned by *Solanum fastigiatum*. Moderate to strong binding to Con-A in the cytoplasm of Purkinje cells. LHQ, Mayer's hematoxylin counterstain, 40X.

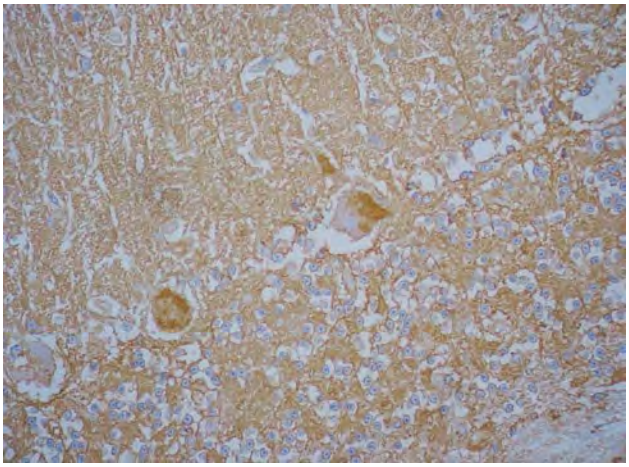

Figure 4. Cerebellum from a bovine naturally poisoned by *Solanum fastigiatum*. Moderate binding to WGA in the cytoplasm of Purkinje cells. LHQ, Mayer's hematoxylin counterstain, 40X.

in other countries where similar clinical manifestations and pathological lesions were observed (Pienaar et al. 1976, Menzies et al. 1979, Bourke 1997, Verdes et al. 2006, van der Lugt et al. 2010). In South Africa, the poisoning by *S. kwebense* (*tettense*) is known as maldronksiekte, which literally means mad- or crazy-drunk-disease (Pienaar et al. 1976).

Electron microscopy studies revealed lipidic inclusions and cytoplasmic membranous and lamellar bodies accompanied by ribosome disaggregation in cerebellar Purkinje cells of cattle intoxicated with *S. fastigiatum* and *S. bonariense* (Riet-Correa et al. 1983, Barros et al. 1987, Verdes et al. 2006). Another typical degenerative histopathologic and ultrastructural change observed is the presence of axonal spheroids that represent swollen myelinated axons (probably by cytoskeletal distortion) filled with electron-dense residual bodies, swollen mitochondria, and an increase in the ratio of axoplasm/myelin (Riet-Correa et al. 1983, Barros et al. 1987, Verdes et al. 2006). It is possible that the neurotoxin(s) contained by these *Solanum* spp. form(s) a complex with lipid material that cannot readily be metabolized by Purkinje cells (Barros et al. 1987) but a direct or indirect role of the neurotoxin(s) on neuronal protein synthesis and/or axonal transport was not discarded from playing a role in the pathogenesis of these cerebellar cortical degeneration (Verdes et al. 2006).

In an experimental reproduction of the disease, specific lectins reacted strongly with stored material in affected Purkinje neurons (Paulovich et al. 2002). Although the toxic principle responsible for the intoxication by *S. fastigiatum* is unknown (Riet-Correa et al. 2009), these previous results have suggested that the poisoning induced by *S. fastigiatum* is classified as a glycolipid storage disease. In *S. dimidiatum* and *S. kwebense* (*tettense*), calystegine B_2 appears to be the toxin responsible for the development of the disease (Nash et al. 1993, Burrows and Tyrl 2001). However, swainsonine and/or calystegines were not detected in *S. bonariense* samples (R.J. Molyneux, 2006, personal communication). Additional studies are needed to better characterize the toxic substances present in *S. fastigiatum* and *S. bonariense*.

Induced storage diseases in domestic herbivores are typically related to ingestion of plants. These conditions include swainsonine toxicosis, *Trachyandra* poisoning, *Phalaris* poisoning, Gomen disease, and *Solanum* poisoning (Maxie and Youssef 2007) and can be classified as glycolipid or glycoprotein storage diseases (Alroy et al. 1984, Alroy et al. 1986). Previous studies have demonstrated that lectin histochemical and ultrastructural analyses allow the identification and characterization of the glycoproteins and glycolipids implicated in these disorders (Alroy et al. 1984, Alroy et al. 1986, Driemeier et al. 2000, Cholich et al. 2009, van der Lugt et al. 2010). Lectins are carbohydrate-binding proteins and glycoproteins of non-immune basis that agglutinate cells and/or precipitate glycoconjugates having saccharides of appropriate complementarity (Goldstein and Hayes 1978).

In the present study, accumulated material in the perikarya of Purkinje cells had marked binding affinities for sWGA, Con-A, and LCA, indicating β-(1-4)-D-N-acetyl-glucosamine, α-D-mannose and α-D-glucose, and D-mannose and D-glucose residues, respectively, and impairment of the function of lysosomal enzymes. Furthermore, D-N-acetyl chitobiose and N-acetyl lactosamine residues, indicated by WGA binding, were detected with appreciable intensity. These findings partially conform to those observed in the cerebellum of cattle poisoned experimentally by *S. fastigiatum* (Paulovich et al. 2002) and spontaneously by *S. kwebense* (*tettense*) (van der Lugt et al. 2010). However, β-D-galactose residues were detected only in the experimental cases previously reported (Paulovich et al. 2002). This finding differs from the current study. It is surmised that this difference is associated with the period of ingestion of the plant since many naturally poisoned cattle included in the present study grazed *S. fastigiatum* or *S. bonariense* over extended and undetermined periods.

Lectin-binding patterns observed in affected Purkinje cells of the present investigation are similar to those detected in plant-induced α-mannosidosis (including poisonings by plants of the genera *Swainsona*, *Oxytropis*, *Astragalus*, *Sida*, and *Ipomoea*), but in the last condition there is additional vacuolization in pancreatic, liver, and kidney epithelial cells (Alroy et al. 1984, Alroy et al. 1985, Driemeier et al. 2000, Armién et al. 2007, Cholich et al. 2009). In addition, these degenerative lesions also occur in neurons of the medulla oblongata and pons (Cholich et al. 2009).

To our knowledge, this is the first time the lectin histochemical aspects of spontaneous cases of poisoning by *S. fastigiatum* var. *fastigiatum* and spontaneous and experimental cases of poisoning by *S. bonariense* in cattle have been characterized and compared. The results of this study show that in all

the cases evaluated in cattle, the pattern of lectin binding is similar to that observed in naturally occurring or experimental reproduction of cerebellar cortical degeneration induced by Solanaceous species in cattle. Further studies are needed to define the specific material stored in accumulated lysosomes in Purkinje cells and the potential role of a distorted cytoskeleton in axonal transport alteration suggested by this cerebellar cortical degeneration in cattle (Verdes et al. 2006).

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