The Safety of Adherus Dural Sealant When the Hydrogel or its Extracts are Placed in Direct Contact with Neurological Tissue

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SUMMARY: To demonstrate the safety of Adherus Dural Sealant when in direct contact with neurological tissue, two neuro-compatibility evaluations were performed. During the first portion of the assessment, Adherus Dural Sealant was evaluated for the potential to cause local irritation or toxicity when implanted within the brain parenchyma. A cannula was used to implant pieces of Adherus Dural Sealant or a comparative control article into the brain parenchyma in test animals. Examinations for clinical signs of disease or abnormality were performed daily and neurological assessments were conducted prior to treatment and at days 4, 14, and biweekly thereafter for up to three months. At 1 week and three months after implantation, four animals per treatment group were euthanized. The brain and proximal portion of the cervical spinal cord were dissected and removed. No neurologic deficits were noted and no adverse reactions were observed for any of the test sites at explant. There was no histological evidence of local (implant site) or distant neurotoxicity associated with the test article implanted within the parenchyma. In the second portion of the assessment, extracts of Adherus Dural Sealant were also evaluated following injection of the prepared extracts or control solutions into the lateral ventricle (ICV) and the cisterna magna (CM) of the brain of a rat. Detailed health examinations and neurologic assessments were conducted at pre-specified intervals. At 4 days and 2 weeks following injection, half of the animals from each cannulation type and treatment group were euthanized and necropsy performed. The microscopic evaluation of the tissues revealed no evidence of a treatment related toxicity. In conclusion, under the conditions of this study, there was no evidence of inflammation or neurotoxicity from Adherus Dural Sealant or its extracts when placed in direct contact with neurological tissues of the rat.

INTRODUCTION

Following cranial or spinal procedures that involve a dural defect, the dura is often closed with suture and/or duraplasty materials. In conjunction with these closure techniques, the application of Adherus Dural Sealant ensures a strong, dimensionally stable, watertight barrier against the egress of cerebrospinal fluid (CSF) and ingress of blood and other products until the dura heals.[1,2,3,4,5]

Since the product is applied as an in-situ polymerizing spray to incised or otherwise compromised dura, there is the possibility that the product may come into direct contact with neurological tissues. We previously examined such a situation during pre-clinical testing of Adherus Dural Sealant in a canine cranial durotomy repair model. In that study, a 2 cm durotomy was closed with three loosely-placed, interrupted sutures leaving a 2 mm dural gap which allowed direct contact with and in situ polymerization of Adherus Dural Sealant on the

cerebral cortex. At necropsy and during histological evaluations there was no evidence of a local inflammatory response or neurotoxicity effect and no evidence of dura-cortex adhesions related to the sealant.[3]

The studies described here further investigate the neurological tissue response produced by Adherus Dural Sealant following either direct implantation of the sealant within the cerebral cortex or the injection of the extracts from the sealant into the lateral ventricle (ICV) and the cisterna magna (CM).[6]

MATERIALS and METHODS

Adherus Dural Sealant (HyperBranch Medical Technology, Inc.) is a synthetic degradable hydrogel sealant designed for use as an adjunct to standard methods of dural repair, such as sutures, to provide watertight closure. Hydrogel films of Adherus Dural Sealant for either direct parenchymal implantation or extraction were prepared by reconstituting the activated polyethylene glycol (PEG) and polyethyleneimine (PEI) components with their respective reconstitution buffers, withdrawing the resulting solutions into the 5 mL syringes and coupling the syringes within a Micromedics Applicator Kit according to the Instructions for Use. The sealant was dispensed by applying pressure to the middle of the syringe plunger, forcing the solutions through applicator and out the mix tip.

The comparative control article for the parenchymal implant study, DuraSeal Sealant System (Confluent Surgical, Waltham, MA), was prepared according to the manufacturer's Instructions for Use.

Parenchymal Implant Study

For this study, the test and comparative control articles were polymerized to a depth of approximately 1 mm on a piece of Teflon. Following at least a 10 minute cure time, core samples of each hydrogel were taken using an 18 gauge blunt tipped needle. The needle, loaded with a hydrogel plug, was then inserted into a stereotactic device and implanted in the parenchyma at the target site (Figure 1). The hydrogel plugs were expelled using a stylet.

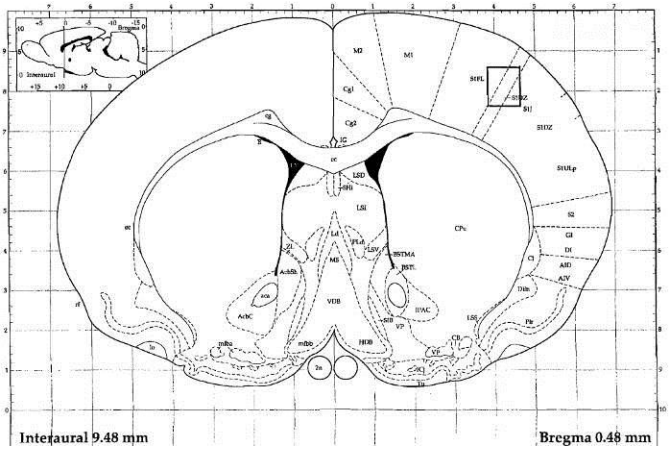

Figure 1 Schematic of rat brain with a box depicting the targeted implantation site 0.48 mm anterior and 4.2 mm lateral to Bregma.

ICV and CM Extract Injection Study

Adherus Dural Sealant extracts were prepared according to ISO 10993-12 guidelines (Biological evaluation of medical devices—Part 12: sample preparation and reference materials, Geneva, Switzerland) by spraying the precursor solutions into a sterilized vessel and allowing the hydrogel to cure. Once cured, the hydrogel was placed in 0.9% sodium chloride solution (Hospira, Inc.) at a ratio of 4 g of test article per 20 mL of 0.9% sodium chloride. The hydrogel was incubated at 37°C to 39°C for 72 hours and subsequently removed to leave the extract solution. The resultant extract solution and 0.9% sodium chloride solution, to serve as the control, were dosed within 24 hours of completing the extraction.

Surgical Procedures

The studies were conducted at Northern Biomedical Research, Inc, in accordance with the

United States Food and Drug Administration (FDA) Good Laboratory Practice Regulations (GLP) (21CFR Part 58), the Japanese Ministry of Health, Labor, and Welfare (MHLW) Good Laboratory Practice Standards Ordinance 21, and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice [C (97) 186/Final].

Parenchymal Implant Study

Sixteen male albino rats (Charles River Laboratories, Portage, Michigan) were used for this study. The animals were randomized into two groups as shown in the Table 1. Four animals per group were sacrificed at each necropsy interval. The rats were approximately 9-10 months old and weighed 310 to 363 grams.

Group	Treatment	Number of Animals / Necropsy Interval	
		1 Week	3 Months
1	Comparative Control (DuraSeal Dural Sealant)	4	4
2	Adherus Dural Sealant	4	4

Table 1 Treatment groups and number of animals at each necropsy interval for the parenchymal implant study.

At implantation, the animals were placed into an induction chamber and anesthetized with 3-5% halothane and 1 L/min oxygen. Once anesthetized, the surgical site was prepared and the animals were moved to surgery where they were maintained on a mixture of 1-3% isoflurane and 1 L/min oxygen. Anesthetic gases and mixtures varied as required for each individual animal. With the animals secured in a stereotaxic frame, a cranial incision was made (Figure 2A). Next a craniectomy was made 0.48 mm anterior and 4.2 mm lateral to Bregma (Figure 2B). Adherus Dural Sealant or DuraSeal (~ 1 mm^3) was implanted in the cerebral cortex at the target site using an 18 gauge blunt-tipped needle such that the implant was placed 1.4 mm below the

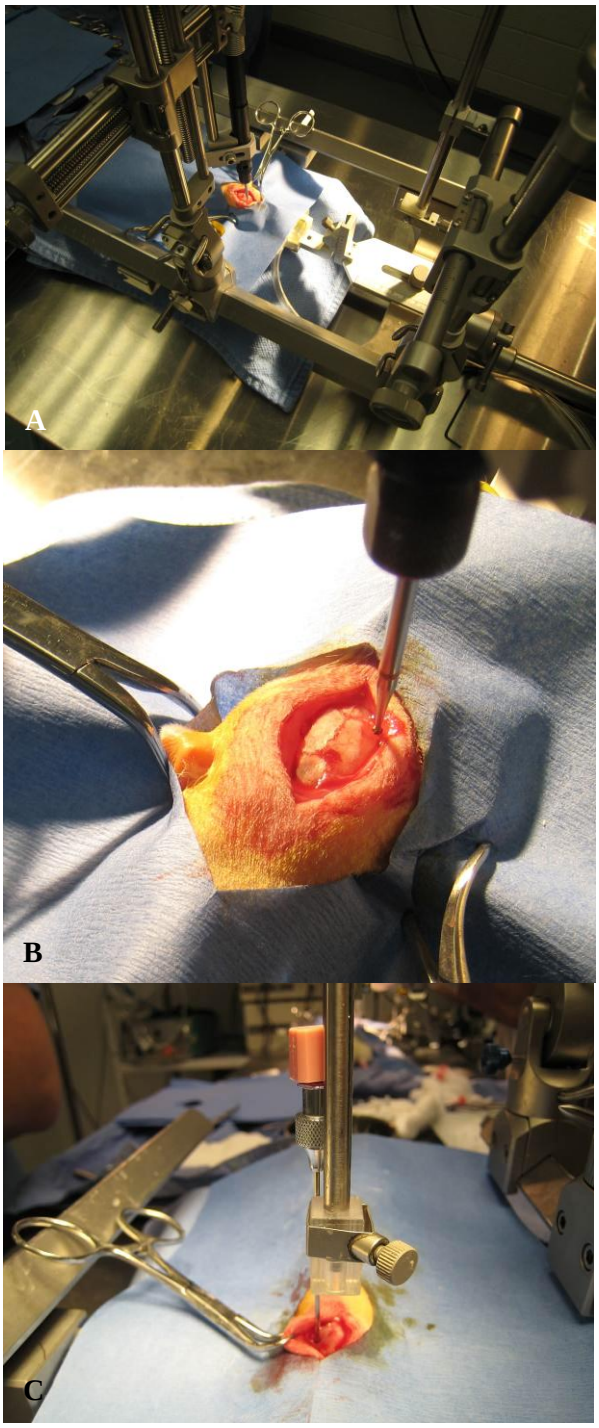

Figure 2 Images from surgery depicting the implantation of hydrogel into the brain parenchyma. First, the animals were placed in the stereotactic frame and a cranial incision was made as shown in Figure 2A. The drill was then positioned at the target site coordinates based on Bregma, which was used as the zero point as shown in Figure 2B. An 18 gauge blunt tipped needle was then inserted at the target site coordinates to 1.4 mm below the surface of the skull and a stylet was used to push the hydrogels out of the needle as shown in Figure 2C.

surface of the skull (Figure 2C). The coordinates of the implantation site were recorded and remained constant for all implants. The craniotomy was covered with dental acrylic and the skin incision was closed with sutures. The animals were recovered from anesthesia, provided butorphanol, IM, and a postsurgical antibiotic, ceftiofur sodium, IM.

Clinical signs were recorded beginning three days before surgery and once daily post-surgically throughout the study period. The animals were observed for signs of clinical effects, illness, and/or death. Body weights and food consumption were monitored weekly. Neurological assessments were performed once before surgery, on Day 4 and bi-weekly after surgery during the study. The animals were removed from cages and placed on a table top where proprioception, motor function and righting were evaluated.

At the designated necropsy intervals, the animals were anesthetized with CO_2. Prior to perfusion with fixative, approximately 150 µL of CSF was obtained via needle puncture into the cisterna magna. Samples were analyzed for chemistry parameters and protein. Following CSF collection, the animals were perfused via the left cardiac ventricle with a heparinized, 0.001% sodium nitrite saline wash followed by 10% neutral buffered formalin fixative. Once the animals were perfused, the brain and spinal columns were removed and placed in 10% neutral buffered formalin.

ICV and CM Extract Injection Study

Twenty-four male albino rats (Charles River Laboratories, Portage, Michigan) were used for this study. The animals were randomized into four groups as shown in Table 2. Three animals per group were sacrificed at each necropsy interval. The animals were approximately 9 weeks old and weighed 290 to 345 grams.

At dosing, the animals were placed in an induction chamber and brought to a surgical plane of anesthesia with a 3-5% halothane and 1 L/min oxygen. Once anesthetized, the surgical site was prepared and the animals were moved to surgery where they were maintained on a mixture of 1-3% isoflurane and 1 L/min oxygen. Anesthetic gases and mixtures varied as required for each individual animal. The animals were secured within a stereotaxic frame and a cranial incision

Study Design

Group	Treatment[a]	Location	Total Number of Animals	Number of Animals Per Necropsy Interval	
				Day 5 (4 day)	Day 15 (14 days)
1	Control (0.9% sodium chloride)	ICV	6	3	3
2	Control (0.9% sodium chloride)	CM	6	3	3
3	Adherus Extracts	ICV	6	3	3
4	Adherus Extracts	CM	6	3	3

[a]Twelve animals received 22 µl of the control or test article into the left lateral cerebral ventricle (ICV). The other 12 received 19 µl injections of the control or test article into the cisterna magna (CM).

Table 2 Treatment groups and number of animals at each necropsy interval for the ICV and CM Extract Injection Study.

was made. For the ICV injections, a craniectomy was performed and the test or control article was injected at a volume of 22 µL into the left lateral ventricle. The craniectomy was covered with dental acrylic. For the CM injections, blunt dissection occurred to expose the CM, a stab incision was made in the dura with a needle and test or control article was injected at a volume of 19 µL into the cisterna magna. Following the injection, the musculature and skin over the injection site was closed with sutures. The animals were recovered from anesthesia, provided butorphanol tartrate, IM, 0.05 mg/kg for analgesia, and 5.0 mg/kg of a post-surgical antibiotic ceftiofur sodium, IM.

Clinical signs were recorded beginning three days before surgery and once daily post-surgically throughout the study period. The animals were observed for signs of clinical effects, illness, and/or death. Body weights and food consumption were monitored weekly. Neurological assessments were performed once before surgery and at 1, 2, 4 and 14 days post-surgery during the study. The animals were removed from cages and placed on a table top where proprioception, motor function and righting were evaluated.

At the designated necropsy intervals, the animals were anesthetized with CO_2. Prior to perfusion, approximately 150 µL of CSF was obtained using a needle puncture into the cisterna magna. Samples were analyzed for chemistry parameters and protein. Following CSF collection, the animals were perfused via the left cardiac ventricle with a heparinized, 0.001% sodium nitrite saline wash followed by 10% neutral buffered formalin fixative. Once the animals were perfused, the brain and spinal columns were removed and placed in 10% neutral buffered formalin.

Histopathology

The brain, with implant or injection sites, and spinal cord were embedded in paraffin and stained with hematoxylin and eosin (H&E). Histopathologic evaluations of the brain, spinal cord and particularly the implant or injection sites were performed to assess any tissue response.

Parenchymal Implant Study

If a defect in the cerebral cortex was noted during microscopic examination and was sufficiently deep to measure, the approximate width of the defect was measured at the time a digital image of the defect was produced. The defects were measured to give a relative size reference. As it cannot be guaranteed that each defect will be sectioned at its greatest width, true quantitative analysis to compare defect sizes between the two groups is not possible. Images were produced and the measurements were made using Olympus DP2- BSW™ imaging software.

ICV and CM Extract Injection Study

The brain was sectioned into eight full coronal sections. These sections included at a minimum the following brain regions: neocortex (including frontal, parietal, temporal and occipital cortex), paleocortex (olfactory bulbs and/or piriform cortex), basal ganglia (including caudate and putamen), limbic system (including hippocampus and cingulate gyri), thalamus/hypothalamus, midbrain regions (including substantia nigra), cerebellum, pons and medulla oblongata. Olfactory lobes were examined when present on the submitted brain tissue. Transverse and oblique sections of cervical, thoracic and lumbar spinal cord were also obtained.

RESULTS

Clinical and Neurological Evaluations

All animals recovered from the surgical procedures, the surgical sites healed as expected

and the animals remained neurologically intact throughout the course of the study. Furthermore, the application of Adherus Dural Sealant, the extracts of Adherus Dural Sealant or the control articles produced no adverse test article-related effects in clinical observations, body weight, food consumption, clinical pathology parameters or CSF chemistry and protein parameters during this study. There were no test article related gross macroscopic lesions observed by visual inspection at any of the necropsy intervals.

Histopathology

Parenchymal Implant Day 8 Sacrifice

There were no histopathologic lesions observed that could be directly attributed to the test articles. All changes in the brain (gliosis, hemorrhage, necrosis, macrophage infiltrates and mineralization) were due to the mechanical trauma induced by the process of implanting the hydrogel samples in the cerebral cortex above the caudate/putamen area.[7,8,9,10] Similar baseline findings were observed with the implant procedures utilized in this study. Microscopic changes in the meninges and cerebral cortex were similar between the comparative control and Adherus Dural Sealant-treated animals.

The sizes of the cortical defects at the implant site were measured (Figure 3). There were no changes in the brain away from the implantation site. All changes at the implantation site of either the control or the test article were also consistent with what would be expected to occur following the mechanical trauma induced by the implantation of 1 mm^3 of material.[7,8,9,10]

The presence of hydrogel was noted at the implant site in three out of four DuraSeal-treated animals and two of four Adherus Dural Sealant-treated animals. The hydrogels were visible as a basophilic (purple) material without any cellular structure. A small amount of mineralized tissue debris, which is invariably observed with minor tissue trauma, was also noted in these specimens. Mineralized tissue debris looked similar to the test article or comparative control but was distinguished as having a more coarsely granular appearance.

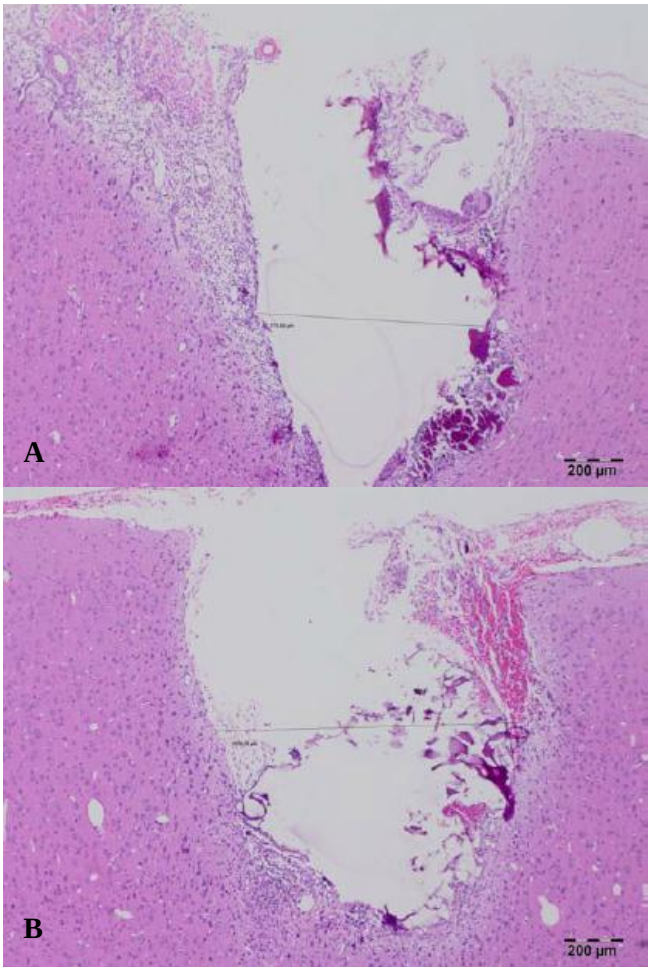

Figure 3 Representative photomicrographs of histology slides from Control (A) and Adherus Dural Sealant (B) at 8 day necropsy (original magnification x 4). Figure 3A shows mineralized necrotic tissue (deep purple fragments on the right side of the defect). The meninges have minimal infiltrates of macrophages and neutrophils. The defect measures 780 microns. Figure 3B shows minimal reaction in the cerebral cortex at the implantation site. The defect measures 1030 microns. Bar = 200 µm.

Parenchymal Implant 3 Month Sacrifice

There were no test article related microscopic lesions. All microscopic changes (gliosis, hemorrhage, necrosis, macrophage infiltrates and

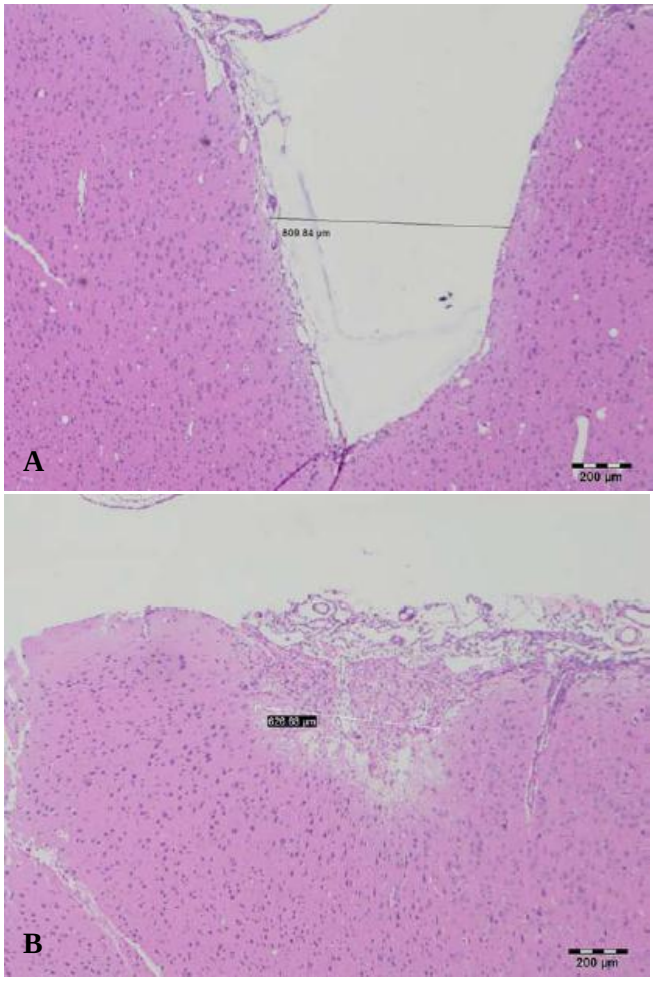

Figure 4 Representative photomicrographs of histology slides from Control (A) and Adherus Dural Sealant (B) at 3 month necropsy (original magnification x 4). Figure 4A shows a cortical defect with minimal reaction. The defect measures 810 microns. Figure 4B shows a small cortical defect filled with reactive (hypertrophied) meningeal cells. The defect measures 627 microns. Bar = 200 µm.

mineralization) were again consistent with what would be expected from the mechanical trauma that results from the implantation of 1 mm^3 of material in the cerebral cortex above the caudate/putamen area.[7,8,9,10] The implant site was identified in three of four Adherus Dural Sealant-implanted animals and three of four DuraSeal-implanted animals (Figure 4). Three months following implantation, the implant sites were not readily apparent at trimming.

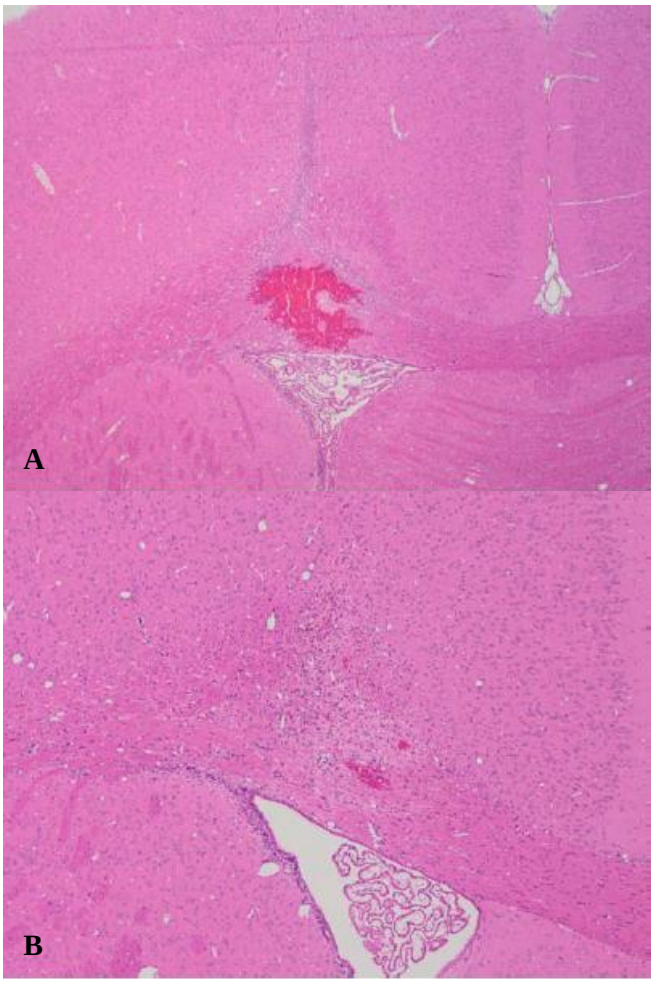

Figure 5 Representative photomicrographs of histology slides from animals at 5 day necropsy that received Control (A) or Adherus Dural Sealant extracts (B) injected into the ICV (original magnification x 4). Figure 5A shows a focal area of hemorrhage along the injection track just above the lateral ventricle. Pointing away from the area of hemorrhage is an area of increased cellularity; this is the injection track. Figure 5B shows a focal area of slight hemorrhage and increased cellularity, probably due to increased glial cells and macrophages, just above the lateral ventricle.

Slight gliosis at the implant site consistent with local mechanical trauma was observed in both treatment groups.

Meningeal changes including lymphocyte infiltrates, hypertrophy of meningeal cells, and hemosiderin (blood breakdown product) were similar between the comparative control and test article implanted group.

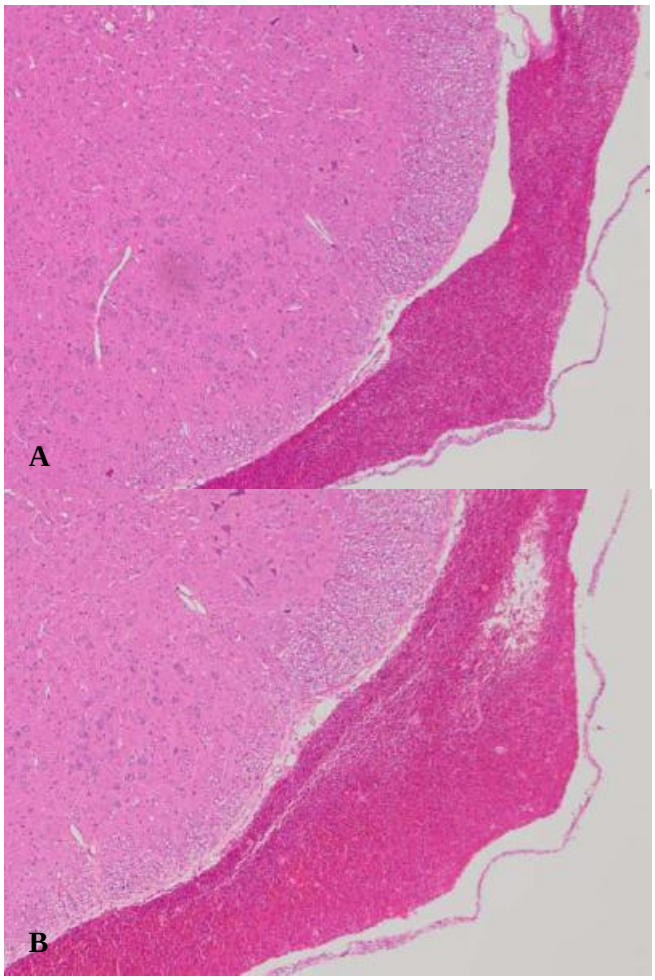

Figure 6 Representative photomicrographs of histology slides from animals at 5 day necropsy that received Control (A) or Adherus Dural Sealant extracts (B) injected into the CM (original magnification x 4). Figure 6A shows the brain (medulla oblongata) to the left and blood in the subarachnoid space (meninges) to the right. Figure 6B also shows the brain to the left and the subarachnoid space filled with blood on the right. The presence of this blood is not unusual following a cisterna magna injection.

No evidence of the comparative control or test article was noted in any of the animals at the three month sacrifice. A slight amount of mineralized tissue debris, consistent with what is typically observed with minor tissue trauma, was noted in two of the Adherus Dural Sealant implanted animals. This mineralized tissue debris was consistent with observations noted in both treatment groups at the Day 8 sacrifice.

ICV and CM Extract Injection – Day 5

There were no histologically significant differences between control and the Adherus Dural Sealant extract-treated brains or spinal cords.

In the control and Adherus Dural Sealant extract-treated ICV brains, hemorrhage in the meninges, injection site and/or in the ventricular system was present in all animals consistent with the recent (4 day prior) lateral ventricle injection. Other minor microscopic lesions, including hypertrophy of endothelial cells, slight necrosis at the injection site, slight gliosis at the injection site and macrophages in the meninges/injection site were also present in controls and the test article-treated animals (Figure 5).

There were also minimal microscopic lesions in the animals that received a cisterna magna injection. Specifically, injection-induced hemorrhage in the meninges (brain or spinal cord) and/or ventricular system was noted in the control and Adherus Dural Sealant extract-treated animals (Figure 6).

ICV and CM Extract Injection – Day 15

There were no histologically significant differences between control and Adherus Dural Sealant extract-treated brains or spinal cords.

Hemorrhage in the meninges (brain and spinal cord) and ventricular system, meningeal cell hyperplasia, macrophages and gliosis at the injection site, endothelial cell hypertrophy and hemosiderin at the injection site were seen in control animals and Adherus Dural Sealant extract-treated animals in equal degrees (Figure 7). A single Adherus Dural Sealant extract-treated animal had a few neutrophils in the brain at the injection site which were likely the result of mechanical manipulation and were not thought to be of biologic relevance.

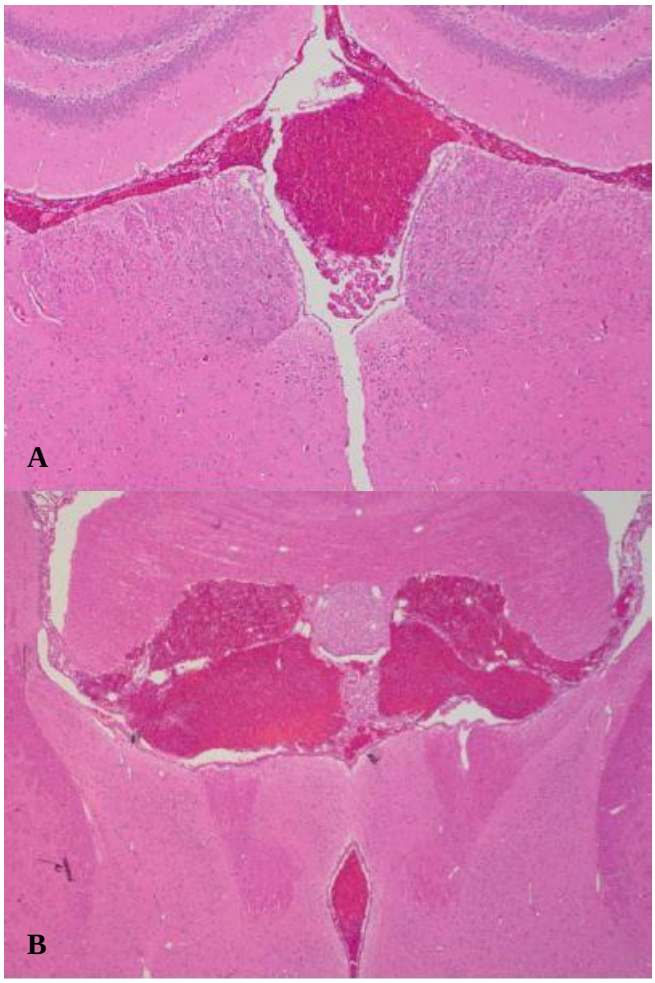

Figure 7 Representative photomicrographs of histology slides from animals at 2 week necropsy that received Control (A) or Adherus Dural Sealant extracts (B) injected into the ICV. Figure 7A shows blood filling the lateral ventricles (original magnification x 10). Figure 7B shows the lateral ventricles and the third ventricle filled with blood (original magnification x 2).

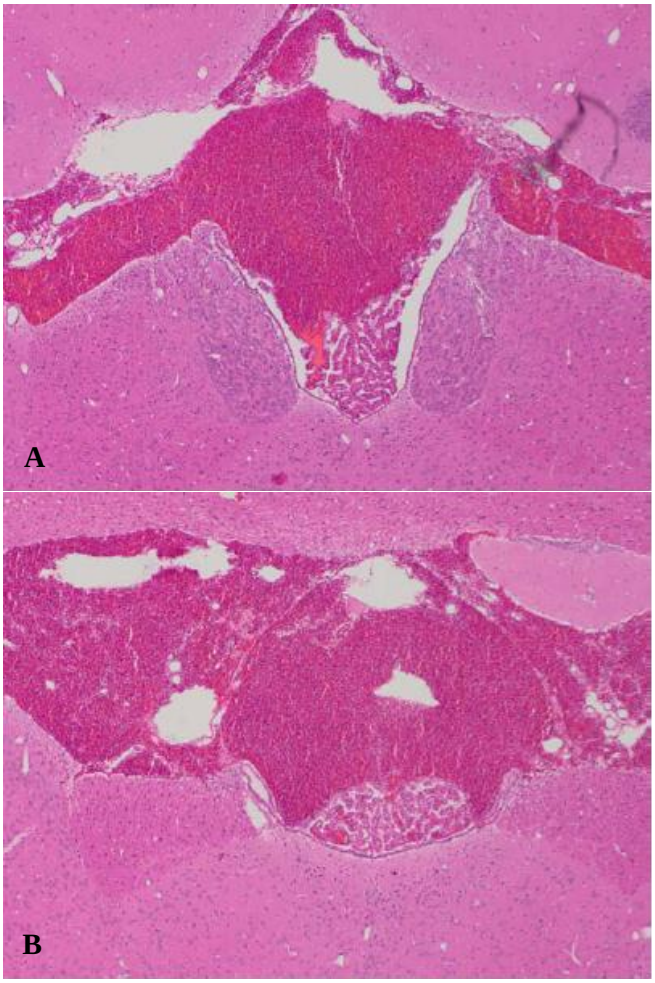

Figure 8 Representative photomicrographs of histology slides from animals at 2 week necropsy that received Control (A) or Adherus Dural Sealant extracts (B) injected into the CM (original magnification x 4). Figure 8A shows the lateral ventricles are filled with blood. Figure 8B also shows the lateral ventricles are filled with blood. Again, the presence of this blood is not unusual following a cisterna magna injection.

There were very few microscopic lesions in the animals that received a cisterna magna injection. Hemorrhage in the meninges (brain and spinal cord) and/or ventricular system were the only morphologic changes noted in the control or Adherus Dural Sealant extract-treated animals (Figure 8).

DISCUSSION

In the analyses described here, Adherus Dural Sealant implanted in neurologic tissue was well tolerated. In addition no evidence of a toxic leachable component was observed.

No clinically significant differences were noted between Adherus Dural Sealant or its extracts and the control materials. No test article-related changes in hematology, serum chemistry or coagulation parameters were noted throughout the studies. There were no biologically relevant differences in body weights or body weight changes between the two groups. Food consumption was within the normal expected range for all animals. CSF analysis showed no

biologically relevant differences between treated and control animals. All animals in all groups were observed to maintain normal neurological function throughout the study.

During histopathological examination of the rats implanted with hydrogel, Adherus Dural Sealant was noted at the implant site at the day 8 sacrifice but was not noted in any animals at the 3 Month sacrifice. This observation confirms previous studies suggesting Adherus Dural Sealant degrades over approximately 90 days. All microscopic changes were considered to be secondary to the mechanical trauma of implantation and the resultant tissue healing process. This opinion is based on reports of similar observations noted in previous implantation studies.[7,8,9,10] The implantation of 1 mm^3 of Adherus Dural Sealant within the cerebral cortex in rats produced no evidence of local inflammation or neurotoxicity. There were also no histopathological changes in the brain or spinal cord in any area away from the implantation site.

Similar histopathological observations were noted in both the Adherus Dural Sealant extract-treated and control animals that received injections in the lateral ventricle or cisterna magna. Again, minor findings observed were thought to be related to the procedure itself. Otherwise, there were no histologically significant differences between the two groups and there was no evidence of neurotoxicity or any type of extract-related inflammatory response.

CONCLUSIONS

After direct implantation of Adherus Dural Sealant into the cerebral cortex, local tissue response was thought to be consistent with what would be expected following the mechanical trauma of implantation. Furthermore, the injection of extracts of Adherus Dural Sealant into the lateral ventricle or cisterna magna did not cause gross or microscopic lesions different from the injection of saline.

In conclusion, the authors have observed that in the biological systems examined, Adherus Dural Sealant appears to behave as a non-toxic, degradable, space occupying mass.

DISCLOSURE

Dr. Asher is a compensated consultant to HyperBranch Medical Technology, Inc. and has received compensation in the form of consultant fees and stock options.

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