

A COMPARISON OF DIFFUSE BRAIN INJURY IN THE NEWBORN AND ADULT PIG

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ABSTRACT

Traumatic brain injury is a leading cause of death and acquired disability in childhood, but the biomechanics of pediatric brain injury are poorly understood. In a porcine model of diffuse brain injury, the anatomic distributions of macroscopic and microscopic injuries were determined following specified inertial (non-impact) rotational loads applied in the axial plane of heads of adult and newborn pigs. The rapid inertial deceleration caused diffuse axonal injury and subdural hematoma in both age groups, but the density and distribution of neural injury was less severe in the immature piglets. These initial data regarding age-dependent injury responses to inertial loads provide a first step towards understanding age-specific mechanisms of head injury.

TRAUMATIC BRAIN INJURY is the most common cause of death in childhood (CDC, 1990). Brain injuries resulting in hospitalization or death occur in at least 150,000 children per year, at a rate of over 200 per 100,000 children. Head injury in infancy results in higher morbidity and mortality than that seen in older children, and it has become increasingly clear that the significant incidence of inflicted injury in the youngest patients is in large part responsible for this difference (Billmire and Myers, 1985; Duhaime et al, 1992; Luerssen et al, 1991; Luerssen et al, 1993). Subdural hematoma (SDH) and diffuse axonal injury (DAI) are the most common findings in serious head injuries in infancy and those associated with non-accidental causes. Determining the conditions that cause these injuries and others in infants and older children will yield more effective prevention, diagnosis, and treatment strategies, as well as providing valuable information regarding injury mechanisms that is required to be able to discern between accidental and inflicted injury etiologies.

The underlying pathology of DAI is the widespread damage to axons in the white matter of the brain, and the level of immediate neurologic impairment is correlated with the extent and severity of axonal damage (Gennarelli, et al 1985). Biomechanical analyses of adult primate and porcine inertial models of

DAI indicate a link between brain material response (tissue strain) and white matter injury (Margulies 1990, Miller 1998). Galbraith (1993) confirmed this finding, showing that uniaxial tensile strain, not stress, caused short- and long-term neural dysfunction in unmyelinated axons.

SDH is often caused by the rupture of the blood vessels in the brain and subdural compartments. In a biomechanical analysis of a primate inertial model for brain injury (Thibault and Gennarelli 1982), SDH was associated with the elongation of the bridging veins beyond their ultimate or rupture strain as the brain moves relative to the skull during a sudden acceleration or deceleration (Meaney 1991). Taken together these studies demonstrate that tissue strain is closely associated with the primary axonal and vascular damage found in DAI and SDH.

Our long-term objective is to use experimental and computational methods to determine specific mechanisms of DAI and SDH in children. Unfortunately, understanding the biomechanics of SDH and DAI in the child has been hindered due to a paucity of animal models for pediatric SDH and DAI, as well as a lack of information regarding measured loads, tissue mechanics and injury thresholds, and computer models to predict mechanisms of injury. The focus of this communication is on the development of a pediatric animal model for SDH and DAI.

Most pediatric animal models for head injury have been used to evaluate the effect of contusional brain injury on the immature rat brain (Prins, 1996; Bittigau, 1998; Grundl, 1994). These models have been particularly useful in characterizing focal injuries, primarily to the gray matter (Prins, 1996; Bittigau, 1998; Grundl, 1994). More recently, Adelson and colleagues (1996) have developed a model of diffuse brain injury in 17 day-old rats. However, unlike humans, rodents have lissencephalic brains and little white matter, as well as a very different maturational sequence from humans. With little white matter, DAI is difficult to characterize in the rat (Meaney et al, 1995; Gennarelli, 1996). Developmentally, the rodent brain has its growth spurt entirely in the postnatal period, whereas humans peak in brain growth at the time of birth (Dickerson and Dobbing, 1967). The small brain size of rodents presents bioengineering limits when designing a device to create an animal model for inertial rotational injury (DAI, SDH). Analyses of the mechanical environment associated with pediatric brain injury must employ animal models that include salient differences between human infant and adult brains, such as differential development of the gray and white matter, regional cerebral metabolism and blood flow, changes in receptor density and distribution at different ages, and changes in biomechanical properties. Therefore, rodent models are limited in their ability to provide data relevant to understanding head injury in human infants and young children (Gennarelli, 1994).

In contrast, piglets provide many advantages in modeling the immature human brain; the overall shape, gyral pattern, and distribution of gray and white matter is similar in pigs and humans. The growth pattern of the postnatal brain is similar to that of human infants; brain weight nearly doubles between the neonatal and juvenile periods, and triples to reach the adult weight (Thomas and Beamer, 1971; Dickerson et al, 1967). In addition, the degree of

myelination, EEG patterns and cerebral blood flow and metabolism in the brain of a 5 day-old pig is similar to the first few weeks of life of a human newborn (Buckley, 1986; Pampiglione, 1971; Wagerle et al, 1996); Corbett, 1990). Visual evoked response remains immature until two weeks after birth (Mattsson, et al, 1978). Investigators of cerebral insult have described the first weeks of life as corresponding to the infant developmental stage (Hoehner et al, 1994; Armstead and Kurth, 1994b; McGowan et al, 1995; Goplerud et al, 1993). Contusional injury has been modeled in pigs (Madsen and Reske-Nielsen, 1997; Madsen, 1990a, Madsen, 1990b), and at our institution, fluid-percussion injury has been modeled in infant and juvenile pigs (Armstead and Kurth 1994a). Based on this detailed information regarding parallels between pig and human development, we used piglets at 3-5 days of age to represent the human infant (<3 months of age) in a porcine head injury model.

The HYGEE apparatus of Penn's Head Injury Center (Thibault and Gennarelli 1982; Meaney, et al 1995; Smith, in review) provides controlled, reproducible inertial rotational loads without impact that are easily incorporated in computational models, facilitating future finite element simulations of pediatric and adult injury responses. Using this apparatus, we have developed an animal model of inertial brain injury that demonstrates SDH and DAI in the immature pig brain. In both adult and immature piglets representing adult and infant humans, we have determined the anatomic distributions of macroscopic (tissue tears, subdural hematoma, intracranial hemorrhage) and microscopic (neurodegeneration, axonal injury, blood-brain barrier breakdown) damage to inertial (non-impact) rotational loads applied to the head. In this communication, we discuss the age and load magnitude dependence of the injury patterns.

METHODS

Adult mini-pigs (N=4) and young (3-5 day, N=4) farm piglets were restricted from food 8 hours prior to surgery. Anesthesia was induced with an initial injection of midazolam (500 mg/kg), and thereafter animals received inhalational anesthesia (2% isoflurane) via endotracheal tube. Piglets were tracheostomized to allow for a proper biteplate fit. A rectal thermometer (to measure core body temperature) was inserted, and mean arterial pressure, arterial O₂ and CO₂ were measured using a mean arterial pressure cuff on the hind limb, oxymeter cuff on the tail, and end-expired CO₂ meter. All measurements were periodically evaluated prior to injury and following injury. All protocols were approved by Penn's Institutional Animal Care and Use Committee.

Studies were performed with head rotational acceleration in the axial plane (Figure 1) with the center of rotation in the cervical spine. Animals were subjected to rapid, purely impulsive, non-impact rotations. Brain injury was induced using a well-characterized head rotational acceleration device to impart a rapid, single 110° axial rotation with its center in the cervical spine. To achieve this motion, the animals' heads were secured to a padded snout clamp, which, in turn, was mounted to the linkage assembly of a HYGEE pneumatic actuator (Bendix, Corp) that converts the impulsive linear motion to an angular

(rotational) motion. Separate customized linkage and biteplate assemblies were machined for the adult and pediatric animals to ensure proper fit and center of rotation. Axial angular velocities of 214 to 286 r/s have been used previously in the adult pig producing unconscious periods from 2 hours to over 8 hours (Smith et al, in review). The target rotational load (velocity) parameters to produce moderate injury in the piglets were determined by scaling the lowest load used previously (214 r/s) that produced unconsciousness (2.5 hrs) in the adult. Applying a relationship proposed by Ommaya (1967) with a scaling factor defined as the inverse ratio of the brain masses raised to the one-third power, and using average brain mass of 35 gm and 72 gm in the piglet and adult, respectively, results in a target angular velocity of 272 r/s for the piglet to provide a scaled mechanical load.

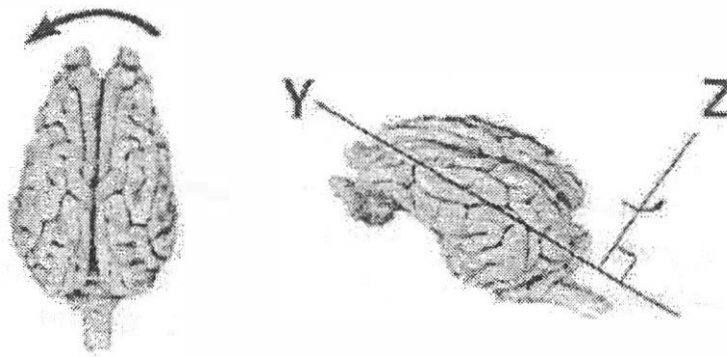


Figure 1 – Axial Rotation (about z axis)

Immediately prior to inducing brain injury in 3 animals in each age group, the animals were taken off anesthesia. Activation of the HYGE device rotated the linkage assembly over the full desired angular excursion within 20 milliseconds. The loading conditions were measured by a piezoresistive accelerometer and an angular rate sensor (ATA, Inc.) attached to the linkage sidearm. Signals were captured using a PC based data acquisition ($f=8\text{kHz}$) and postprocessed using appropriate filtering algorithms (SAE J211). Following acceleration, the linkage was slowly moved back into its original position, the animal's snout removed from the biteplate, and respiratory, cardiac, and neurological status was assessed. If apneic, mechanical ventilation was provided (tidal volume 10ml/kg body weight). Neurologic status was monitored using corneal and pupillary reflex and pinch tests to assess the response to physical stimuli. Once animals responded to the pinch reflex, anesthesia was resumed. One additional pig in each age group served as an uninjured control, and all gross and histological analyses are reported relative to these uninjured control animals.

The animals were euthanized 6-8 hours after injury. The brains were perfusion fixed by transcatheter perfusion with heparinized saline (1 liter, 5000 units heparin per liter), followed by 4% paraformaldehyde. Brains were removed from the cranial cavity and post-fixed overnight at 4°C. The brains

were blocked into 0.5 cm coronal sections for gross examinations and photography. Blocks were cryoprotected in sucrose.

The short post-injury survival period prior to sacrifice minimizes secondary axonal damage due to neurochemical and pathological processes occurring within the brain after injury, to allow us to focus on the areas of primary strain-induced damage. The brains of 3 injured adult and 3 injured immature pigs, as well as 1 uninjured control animal in each age group, were examined for microscopic neural and vascular injury. Sections (40 μ m thick) from the cryoprotected blocks were stained with cresyl violet (Nissl) and evaluated using a light microscope for regional tissue tears in white matter, presence of intracerebral hemorrhage in white and gray matter and overt neurodegeneration (pyknosis) in the gray matter. Microscopic evidence of axonal injury was investigated in the white matter of the brain and brainstem using neurofilament (NF) immunohistochemistry. Reactive axonal changes were determined by identifying the presence of swollen axons and terminal "retraction balls" containing neurofilament protein. Free-floating 40 μ m thick sections cut from cryoprotected blocks using a freezing sliding microtome, were immunostained for 68 kDa (light) and 200 kDa NF (heavy) protein subunits. Sections were incubated with anti-NF antibody (Sigma, clone NR4 for 68 kDa or clone N52 for 200kDa; each 1:400) overnight at 4°C, followed by biotinylated donkey anti-mouse IgG (Jackson, 1:2000). Antibody complexes were detected using avidin-biotin-peroxidase (ABC) histochemistry (Vector Labs) and 3,3' diaminobenzidine as chromogen. Omission of primary antibody on selected sections of uninjured and injured pig tissue provided a negative control.

Blood-brain barrier breakdown was assessed using immunoglobulin (IgG) immunohistochemistry. Parallel sections to those used for NF staining were incubated overnight at 4°C with biotinylated donkey anti-swine IgG (Jackson, 1:1000). Biotinylated anti-IgG were detected using avidin-biotin-peroxidase (ABC) histochemistry (Vector Labs) and 3,3' diaminobenzidine as chromogen. Omission of primary antibody on selected sections of uninjured and injured pig tissue provided a negative control.

Evaluation of brain regions included frontal, parietal, and temporal lobes, lateral ventricle, thalamus and brainstem. The entire area of each region was evaluated for specific pathologic profiles which were recorded on schematics. All observations are reported relative to uninjured control pigs matched for age.

RESULTS

In the piglet, peak angular velocities were 237, 257, and 264 r/s resulting in unconscious periods of 5 minutes, 50 minutes, and 4.5 hours, respectively. The mean velocity was 253 r/s and total load duration of 11.8 msec. Three adult animals studied were subjected to angular velocities of 232, 234, and 286 r/s resulting in unconscious periods of 5, 4.5, and over 8 hours respectively. In the adult mini-pigs studied, peak angular velocities in the lower load range (mean velocity of 233 r/s and duration of 13 msec) produced comparable durations of unconsciousness as the piglet.

Immediately following the load, all animals were unconscious. All 3 piglets and 2 adult pigs were apneic, and pupillary, corneal, and pain reflexes

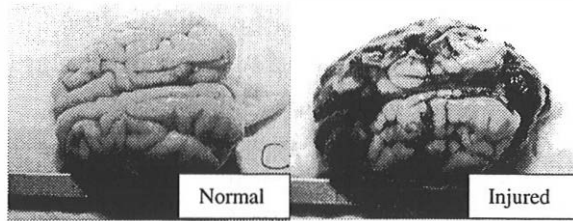


Figure 2 Gross Examination of Pediatric Brain

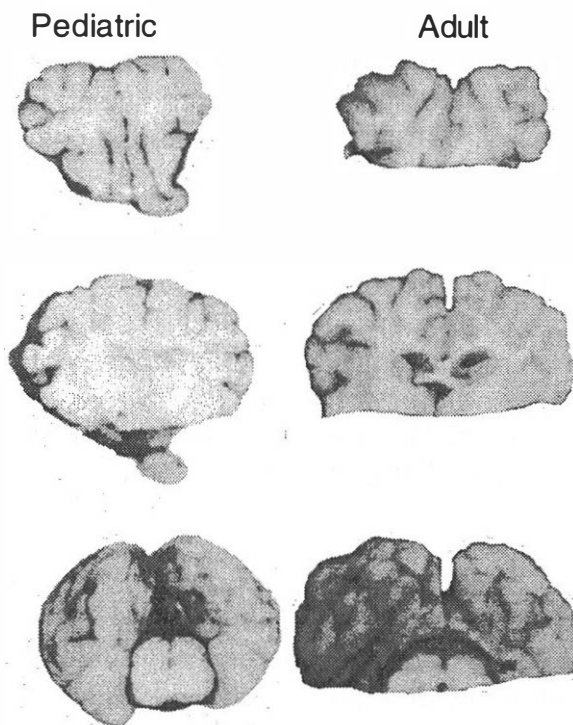


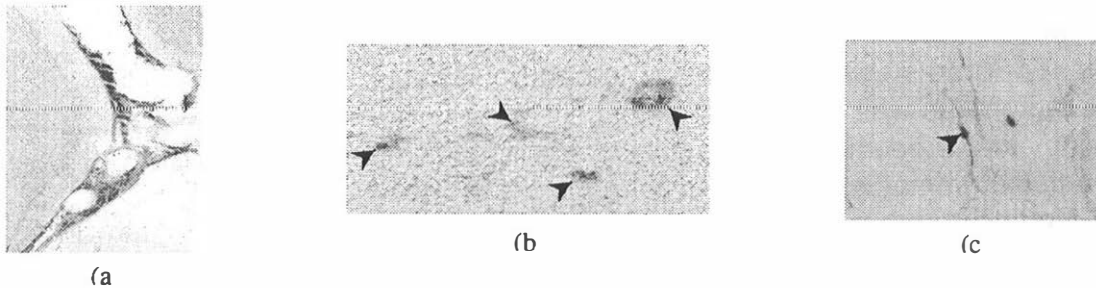
Figure 3 Coronal Brain Sections

were absent following injury. They received mechanical ventilator support until spontaneous breathing was restored. No persistent changes in physiologic parameters (temperature, mean arterial pressure, PO₂, PCO₂) were observed in any injured animal.

In the macroscopic examination of injured brains in both age groups, blood was observed on the surface of the cerebellum, brainstem, and left (leading edge of the brain) frontal and temporal lobes, indicative of subdural bleeding, but was not restricted to the leading edge of the brain (Figure 2). Gross evaluation of paraformaldehyde fixed brain tissue blocks was also similar in adult and immature pigs, with ventricular hemorrhage in the lateral ventricles and an absence of cerebral tissue tears. Frontal lobe cortical contusions and dilation of the ventricles was only observed in the adult pigs (Figure 3).

In brain sections stained with cresyl violet (Nissl), no evidence of neuronal loss (pyknosis) was observed in any region of the injured piglets, but pyknosis occurred in the hippocampus of 2 adult pigs and the cortex of 1 adult pig examined. In both age groups, sub-pial blood was visible over the frontal cortex (Figure 4a), and petechial hemorrhage was evident in the gray and white matter (Figure 4b). No overt evidence of blood-brain barrier disruption (using anti-swine IgG antibody) was observed in the piglet, but occasional reactivity was observed in the frontal subcortical white matter and brainstem of the adult.

Figure 4: Histological Analysis of Brain Injury in the Piglet



Using an antibody targeting light chain neurofilament subunit proteins (68 kDa), axonal swelling (arrowhead in Figure 4c) and occasional retraction bulbs were found along the parasagittal gray/white matter interface in the leading (left) frontal lobe of all adult animals and the 2 piglets with prolonged unconsciousness. Specifically, the piglet unconscious for only 5 minutes had no axonal injury compared with controls, but did have subdural bleeding and intraparenchymal hemorrhages. Interestingly, the damaged axons in the piglet were interspersed with axons that appeared morphologically normal. In addition, the adult pig had intraparenchymal axonal injury in the basal ganglia and caudate nucleus, but these regions were not injured in the piglet. Using the antibody that detects the heavy (200kDa) NF protein subunits, little to no axonal injury was detected in the piglet brain. In contrast, adult observations were identical for both subunits. The distinct subunit responses in the immature brain may reflect age-dependent differences in neurofilament protein

transport and/or differences in NF susceptibility to proteolysis. In both age groups, we observed hemorrhagic and axonal lesions in the brainstem.

The neural and vascular cerebral injury distributions were asymmetric, with a preference for the leading hemisphere in both age groups. Although evidence of tissue injury was observed in each injured brain evaluated, the preliminary nature of these experiments preclude any evaluation of a relationship between load magnitude and tissue injury severity in either age group. However, we observed that the duration of unconsciousness and severity of axonal injury increased with peak angular velocity regardless of age.

DISCUSSION

Children under the age of 3 years of age are particularly vulnerable to non-accidental traumatic brain damage (Duhaime et al, 1998). Macroscopic and microscopic patterns of damage have been described in autopsy tissue (Duhaime et al, 1992) and the spectrum of pathology includes focal contusions, subdural hematomas and, importantly, microscopic evidence of diffuse axonal injury (DAI) such as intra-axonal and terminal swellings, tissue tears and intracerebral hemorrhages. We have previously demonstrated that the severity and location of the axonal pathology in the brains of adult primates or pigs is related to the regional magnitude of tissue deformation (strain) (Margulies et al, 1990; Miller et al, 1998). Specifically, tissue injury occurs in a particular region when maximum strains exceed the tissue's threshold for a particular neural or vascular injury. Regional strains are influenced by brain size, anatomy, and mechanical properties of the tissue, as well as the magnitude of the load (e.g. force, acceleration) experienced by the head. However, understanding how these factors, as well as development, contribute to pediatric head injury remains elusive because of a paucity of age-specific data regarding mechanical properties of immature tissue and its response to specific loads.

In this communication we present an experimental model for DAI and SDH in adult and immature surrogates whose neurological development have been correlated with human adults and infants. Specifically, rapid, inertial (non-impact) rotation of the head of the pig about its axial plane has been observed in both adults and immature pigs to cause immediate periods of coma, accompanied by microscopic evidence of intracerebral hemorrhage, and axonal injury. We observed that the duration of coma, a characteristic of clinical DAI, appeared to correlate with magnitude of the loading conditions in both adult and infant pigs. In addition, in both ages, microscopic brain damage (hemorrhage and axonal injury) was evident. The anatomic distributions of macroscopic injuries (subdural hematoma and hemorrhage), and microscopic injuries (neurodegeneration, axonal injury, blood-brain barrier breakdown) were determined. These data demonstrate that a non-impact rapid inertial deceleration can cause diffuse axonal injury and subdural hematoma in immature piglets similar to that observed in children with severe traumatic head injury. Furthermore, the data suggest that a similar pattern of axonal pathology with subdural hematoma was observed in the immature brain following rotational injury, but with an absence of injury in the basal ganglia and caudate nucleus found in the adult brain. Furthermore, in the regions injured in both

age groups, the density of injured axons was greater in the adult. Taken together these data suggest that axonal injury is less severe in the immature brain. Although the average piglet load was 10% below our targeted scaled mechanical load, the general histological observations of less severe neural injury in the piglet also holds for the comparison between brains from two mechanically equivalent loads: the highest load in the piglet and the lowest in the adult pig. These age-dependent pathology differences at mechanically equivalent applied angular velocities may be the result of different regional strains (due to developmental variations in geometry or constitutive properties), or tissue strain injury thresholds. However, the small number of subjects evaluated (N=3 injured in each age group) precludes a formal analysis of differences in injury severity due to age.

Traditionally, a biomechanical analysis of head injury in the infant and young child assumes that they respond as miniature adults, with identical tissue properties and injury thresholds (Dejeammes, 1984; Duhaime, 1987; Mohan 1979; Stürtz, 1980, Margulies, 1992). Using dimensional analysis, critical inertial loading conditions associated with SDH and DAI were scaled from the adult to the infant as a function of brain mass (Ommaya, 1967). These studies concluded that the lower brain mass of the young child allows the pediatric brain to sustain higher rotational accelerations than its adult counterpart before the onset of injury. However, alterations in tissue composition and mechanical properties can influence the resulting deformations and, in turn, injury patterns within the brain. Recently, Melvin (1995) and Hymel (1998) recognized the important role of material properties in defining age-specific injury mechanisms and thresholds, and emphasized the current lack of age-related material property data within the literature. Specifically, previous studies are limited to quasi-static properties for perinatal skull (McPherson and Kriewall 1980; Kriewall et al, 1981), and recent work from our laboratory characterizing small-deformation properties of porcine infant brain tissue and skull (Thibault and Margulies, 1998; Margulies and Thibault, in review).

We hypothesized that the threshold for histological abnormalities following a non-inertial traumatic insult is dependent on the age/maturation of the neonatal brain and the magnitude of the angular load applied to the head. Based on these early studies in adult and pediatric porcine subjects, we find suggestions of a dependence of injury severity on both load magnitude and age, tentatively confirming our hypothesis. Future studies in both age groups will permit formal statistical analyses of these trends. Furthermore, additional porcine experimental studies in both age groups are necessary to evaluate the validity of biomechanical scaling relationships based on brain mass alone by comparing duration of unconsciousness and overall injury severity between immature and adult animals, matched for supposedly equivalent applied loads. If the scaling relationship does not hold across developmental ages, we will be able to develop a new relationship, incorporating developmental changes in brain properties, geometry, and tissue strain injury thresholds. The early findings regarding age-dependent injury responses to inertial loads presented in this communications begins to address our long-term objective of understanding the unique biomechanics associated with pediatric head injury to

open pathways for enhanced traumatic head injury prevention, detection, and treatment strategies specific to infants and toddlers.

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