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Evidence for infection, inflammation and shock in sudden infant death: parallels between a neonatal rat model of sudden death and infants who died of sudden infant death syndrome

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Abstract

This study compared pathological findings from a neonatal rat model of sudden death with those from 40 sudden infant death syndrome (SIDS) infants collected at autopsy. In the rat model, influenza A virus was administered intranasally on postnatal day 10, and on day 12 a sublethal, intraperitoneal dose of *Escherichia coli* endotoxin; mortality was 80%. Tissue samples from the animals and infants were fixed in formaldehyde, embedded in paraffin, and sections stained with hematoxylin and eosin. Tissues from the SIDS specimens were additionally cultured for bacteria and viruses; post-mortem blood samples were evaluated for signs of inflammation. All sections were examined by a pediatric forensic pathologist familiar with SIDS pathology. Comparisons between the rat model and the human SIDS cases revealed that both exhibited gross and microscopic pathology related to organ shock, possibly associated with the presence of endotoxin. Uncompensated shock appeared to be a likely factor that caused death in both infants and rat pups. Response to a shock-inducing event might have played an important role in the events leading to death. The similarities between the neonatal rats and the human cases indicate that further research with the model might elucidate additional aspects of SIDS pathology.

Keywords

Endotoxin; organ shock; sudden infant death syndrome; SIDS; thymic involution

INTRODUCTION

Sudden infant death syndrome (SIDS) is defined as the sudden death of an infant between 1 month and 1 year of age that cannot be explained following a thorough death-scene investigation, complete autopsy, and review of the clinical history.[1,2] Affected infants appear normal and healthy when they are put to bed, but they die quietly in their sleep. A history of a mild viral illness often precedes death but is not thought to be its cause.[1,3]

While risk factors identified in epidemiological studies do not cause sudden death, they appear to increase susceptibility. Maternal smoking and increased incidence in winter months parallel

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closely susceptibility to infections, particularly those of the respiratory tract. Ethnic groups at increased risk of SIDS also exhibit high incidences of respiratory diseases.[4]

Some autopsy findings observed in SIDS infants remain difficult to explain. Many SIDS infants show characteristic intrathoracic petechiae and liquid blood in the heart.[5,6] These abnormalities could be associated with the individual's efforts to resuscitate or overcome hypoxia, respiratory distress, or suffocation; however, they could also be due to the problems of coagulation that often accompany infection and inflammation. Mast cell degranulation has been documented in some SIDS infants, and release of heparin from the granules might act as an anticoagulative factor.[7,8] While autopsy findings have varied, signs of inflammation and response to infection have appeared out of proportion to pre-existing symptoms.[9,10] Mild thymic involution, a sign that the infant has been responding to excessive stress, indicated by signs of adrenal over activity, has been observed in more than half of SIDS cases.[10]

A useful model of disease should reflect the properties that define the human cases. In the case of a SIDS model, characteristics should include an uneventful history, no obvious explanation for the cause of death, and a narrow window of age of susceptibility. Careful consideration must be given to associations between the human condition and the findings in the model. Insults triggering a response in the model should be plausible and compatible with daily living. They cannot be so severe that they are obviously the cause of the death.

We have developed a model of sudden death in neonatal rodents in which the pathology is similar to that seen in SIDS infants at autopsy. Since many SIDS infants were reported to have a mild viral infection before death, animals were infected with a rat-adapted influenza strain. Two days later, the rats were challenged with a sublethal dose of *Escherichia coli* endotoxin to stimulate an inflammatory response. The majority (80%) of the animals died within 8–10 hours post-challenge with few symptoms prior to death. When given alone, neither the virus infection nor the endotoxin dose was lethal. As previously reported, susceptibility to sudden death in this model was also dependent on the age of the animal.[11,12]

Evidence indicated that the death of the rats was due to rapid systemic shock. Endotoxin is known to lower blood pressure and cause shock.[13] In these animals, tail blood pressure measurements were lower in the animals challenged with both virus and endotoxin than in animals given endotoxin alone. Gross pathological findings (lung petechiae and liquid blood around the heart at necropsy) were consistent with the findings described in infants who died of SIDS. Histopathological lesions (subendocardial hemorrhage, liver necrosis, and mild cortical thymocyte necrosis) also indicated a shock response.[11,12,14]

The concept that shock constitutes a factor in SIDS pathology is not unique.[15,16] Parents often report that their infants were not totally 'normal' in the days prior to a SIDS death.[1,10] Some of these infants were described as 'listless' or 'droopy' with increased sweating at the time of death.[17,18] During infancy, increased sweating is unusual and indicates a response to an abnormal event such as heat stress, infection, vasomotor instability, or cardiovascular shock. These symptoms suggest that SIDS infants are not normal prior to death, and shock might be involved in the events leading to death.[19,20]

This study is a retrospective comparison of pathological findings obtained from a rat model of sudden unexplained death and data previously obtained from SIDS autopsy published in 1999.[10]

MATERIALS AND METHODS

Animal model

Animal samples were acquired from experiments previously described.[11,12] These studies were performed with the approval of the Animal Care and Use Committee at the National Institute of Environmental Health Sciences (Research Triangle Park, NC, USA). Briefly, timed-pregnant Fischer 344 rat dams were allowed to litter in solid bottom plastic cages (one dam per cage) maintained with 12-h light-dark cycles; and allowed food and water *ad libitum*. Within 48 h of birth, rat pups were randomly cross-fostered and litters standardized to 10 pups per dam. Cross-fostering, a common practice in developmental studies, randomizes the effects of mothering and genetics in the population tested.[21]

On postnatal day 10, we administered a 25 μl intranasal dose of a rat-adapted influenza A virus developed from Influenza A/Port-Chalmers/1/73 (H3N2), a gift from Dr Gary Burleson (Burleson Research Technologies, Raleigh, NC, USA).[22] Two days later, influenza-infected animals were given 0.2 mg/kg of endotoxin from *E. coli* (Sigma, St Louis, MO, USA) in a single intraperitoneal dose, and randomly assigned to groups ($n = 5$). Animals were killed 2, 4, 6, or 8 h after administration of endotoxin, and tissues (liver, spleen, thymus, heart, lung, and kidney) were prepared for histological analysis by fixation in 10% (v/v) formaldehyde and embedment in paraffin. The blocks were sectioned at 5–6 mm, and stained with hematoxylin and eosin. The tissue sections were reviewed by a pediatric forensic pathologist familiar with SIDS pathology and compared with histological findings from human infants.

Samples from SIDS infants

The human samples were obtained from a convenience sampling of 40 consecutive autopsies on infants diagnosed as SIDS between November 1994 and October 1998. These infants, aged 1 month to 1 year (mean, 3.6 months) were collected at a referral center in France. They had undergone a comprehensive post-mortem evaluation, recording of clinical history, and death-scene investigation to rule out other possible causes of death. Resuscitation of the infants had been attempted in 26 of the 40 cases; however, temporary cardiac activity occurred in only three. The interval between death and autopsy, was 4 h to 4 days, as described previously.[10] During autopsy, tissues from every organ were fixed in formaldehyde (10%, v/v), embedded in paraffin and stained with hematoxylin and eosin as described above.[10]

Bacterial and viral cultures were taken under rigorous aseptic conditions from the blood, liver, lung, heart, spleen, kidney, meninges, choroid plexus and bladder. Samples from the CNS were not taken if the autopsy was performed > 48 h following death. Bacterial samples were evaluated using specific criteria to rule out commensal flora. Threshold for the pharynx and upper respiratory tract was $> 10^6$ colony forming units (CFU)/ml; urine $> 10^5$ CFU/ml and 10^4 leukocytes/ml. For stools, only enteropathogenic organisms were accepted. In solid organs, infection was diagnosed if the bacterium was grown in two different sites. For blood cultures, the organisms had to be isolated in two different culture bottles. Viral samples obtained from nasal swabs and upper respiratory aspiration were examined using immunofluorescence to determine respiratory syncital virus, influenza, parainfluenza, and adenovirus. Polymerase chain reaction (PCR) techniques were used to determine enterovirus.

These data were then evaluated by a pediatrician, pathologist and bacteriologist for the relevance of the findings. Bacteriological results were interpreted with regard to the location where the micro-organism was obtained, its pathological potential, the infant's age and the usual flora from this site. Criteria for infection were the same as those used for living children.[10] Data comparing the incidence of microbial culture for all SIDS infants, SIDS infants with

negative histological signs of shock, and SIDS infants with positive histological signs of shock were analyzed using Fisher's exact test.

Criteria for assessment of pathophysiological changes

Histological slides from both animal and human subjects were graded for acute thymic involution using criteria corresponding to signs of steroid-mediated stress. This was manifested as the emergence of the 'starry-sky' appearance, a sign of early thymic involution, or macrophages in the thymic cortex with progressive lymphoid depletion.[23] According to these criteria, no visible modification of thymic features occurs in the first 12 h of stress. Grade I corresponds to a stress level seen at 12–24 h; the tissue includes a few cortical macrophages with lymphophagocytosis. Grade II (24–48 h) has numerous cortical macrophages and lymphophagocytosis. Grade III (48–72 h) exhibits more severe cortical lymphoid depletion, shrinkage of medullary areas, and loss of corticomedullary differentiation. At Grade IV (> 72 h), complete lymphoid depletion is observed.

Histological sections were evaluated for any alterations, particularly those suggestive of shock: (i) foci of ischemic fibers and hemorrhage in the heart; (ii) a marbled appearance and/or hemorrhage in the liver; (iii) glomerular retractions and exudation within glomeruli associated with tubular cell necrosis and/or proteinaceous casts in the renal tubules; and (iv) an increase in the numbers of circulating polymorphonuclear leukocytes and fibrin thrombi in capillaries, representing disseminated intravascular coagulation.[10,14]

RESULTS

Evidence of infection in SIDS infants

Among the SIDS cases, micro-organisms were identified in 34 of 40 (85%) sets of specimens tested and evidence of inflammation was found in 12 of 40 (30%) of the samples. *E. coli* was the most commonly isolated species, found in 18 of 40 (45%) infants (Table 1). Other bacterial isolates included: *Staphylococcus aureus* ($n = 3$; 8%); *Enterococcus faecium* ($n = 3$; 8%); *Haemophilus influenzae* ($n = 2$; 5%); and one case each (3%) of *Morganella morganii*, *Salmonella typhimurium*, and *Veillonella* spp. The following viruses were cultured from 13 of 40 (33%) infants: enterovirus ($n = 8$; 20%), respiratory syncytial virus ($n = 2$; 5%), rotavirus ($n = 2$; 5%), and adenovirus ($n = 1$; 3%). In most cases, the presence of the micro-organism was associated with inflammatory changes, polymorphonuclear leukocytes in the circulation and evidence of shock in the organs. The presence of *E. coli* was significantly associated with histological signs of shock ($P = 0.01$; Table 1).

It is important to note that the majority of infants dying of non-bacterial causes in the first year of life do not show signs of bacterial colonization, except in SIDS infants. The odds ratio for finding coliforms in the respiratory tract of SIDS infants is 29 compared to normal live infants.[24]

Evidence of inflammation

For samples from the rat model, signs of inflammation were especially noted in the vessels of the lung and the liver. Historically, interstitial inflammation was observed in the dually challenged rats; there was margination of the leukocytes along the vessels' walls and 'rolling' associated with inflammatory cytokine release. No extravasation of inflammatory cells was observed outside the organs, but minimal connective and adipose tissue in the sections precluded adequate evaluation.

In the samples from SIDS infants, post-mortem C-reactive protein levels (> 10) were observed for 32 of 40 (82%) SIDS cases. Eight of the 32 cases (25%) exhibited elevated blood levels of

C-reactive protein, which is indicative of increased inflammation. Elevated C-reactive protein was associated with positive microbiology in the following 7 of 8 cases: 2/8, one or two bacterial species; 4/8, bacteria plus a virus; 1/8, virus alone. White blood cell counts were available for 29 of 40 (73%) SIDS cases. Values indicating response to infection were evident in 20 of 29 (69%); 15 of 29 (52%) exhibited leukocytosis, and 5 of 29 (17%) showed leukopenia. Both leukocytosis and leukopenia are signs of inflammatory response. Leukopenia in an infant is a particularly ominous sign due to the small storage pool of neutrophils.[25]

The majority of SIDS infants, (32/40; 80%) exhibited no symptoms prior to death that would account for the severity of their condition; 15 of 40 (38%) had only minor symptoms, and 17 (43%) had symptoms such as mild fever, rhinitis, or coughing. The remaining 8 (20%) showed symptoms that should have been recognized as 'alarm symptoms', including drowsiness, anorexia, high fever, previous apparent life-threatening events, and indications of gastroenteritis.

Signs of stress

No signs of stress were detected in the rat thymus of control animals. At 2-h post-endotoxin challenge, 4 of 5 pups treated with both agents exhibited Grade I signs of thymic involution. By 4 h, 2 of 5 animals were Grade I, and 3 of 5 were Grade II. At 6 h and 8 h, all animals demonstrated Grade-II involution. Among die 40 SIDS infants, 25 (63%) showed signs of stress, indicated by mild-to-moderate thymic involution. Among these, 19 (48%) were Grade I, and 6 (15%) were Grade II showing 'starry-sky' appearance.

Signs of shock

At 6-h post-endotoxin administration, the dually challenged rat pups were cool to the touch, and adequate cardiac blood samples were difficult to obtain. They were hypotensive by tail-cuff measurement (data not shown). Following cardiac puncture at 6 h, they continued to bleed without clotting, suggesting coagulative abnormalities commonly seen in septic shock. At 8 h, the animals were quietly sleeping but lethargic when aroused. Upon necropsy, milk was found in their stomachs, indicating that they had continued to nurse up to the lime of death.

Prior to endotoxin treatment, megakaryocytes are located in the spleens of untreated animals. This is not changed by influenza administration. Megakaryocytes began to appear in the liver 2 h after administration of endotoxin, and their numbers peaked by 4 h. An inverse relationship appeared to exist between the number of megakaryocytes in the spleen and their presence in the liver over time.

In the animals, other evidence of shock in the liver included blood-free areas intermingled with areas of congestion. This abnormality resulted in a 'marbled' appearance at 2 h after injection of endotoxin and progressed to mixed areas of necrotized and hemorrhagic liver accompanied by increased polymorphonuclear leukocyte infiltration (Table 2).[14] No thrombi were observed in the capillaries to indicate disseminated intravascular coagulation.

For nearly all of the animals, maximum changes were noted 6 h after administration of endotoxin (Table 3). Microscopic evidence of shock in the heart included the presence of sparse hypereosinophilic myocytes with condensed and shrunken nuclei. Macroscopic signs of shock, (pale liver and pale kidneys) were seen in most of the animals (Table 2).[12,14]

In some of our earlier experiments, animals were not euthanized prior to death but were allowed to expire naturally. Post-mortem necropsies on these animals revealed additional similarities to the SIDS autopsies – renal-tubule degeneration, red-pulp congestion of the spleen, and lung congestion.[12,26]

In the SIDS infants, microscopic signs of shock included: megakaryocytes in the circulation outside the lungs; sparse hypereosinophilic myocytes with condensed and shrunken nuclei; fibrin thrombi in the capillaries disseminated intravascular coagulation; and, in kidney sections, the presence of glomeruli retractions and exudation within glomerular capsules associated with tubular-cell necrosis and/or proteinaceous coagulation in the lumen of the kidney (Table 2).[14,27,28]

Histological examination of various SIDS tissues showed that one or more signs of shock were present in 22 of 40 (55%) SIDS infants. Macroscopic signs of shock included: marbled skin; marbled liver; and/or pale kidneys. These were observed in 12 of 40 (30%) SIDS infants. Macroscopic and/or histological signs of shock were apparent in 28 of 40 (70%) of the SIDS infants (Table 4).

Of the 22 infants with histological signs of shock, 20 (91%) had positive microbial cultures. Thirteen were positive for one or more pathogenic bacteria (59%), two for viruses only (9%), and six for both (27%). *E. coli* was the most common species (14/22; 64% of infants; Table 1). Eight of the infants (36%) with signs of histological shock exhibited diffuse inflammatory foci outside organs. Fifteen of the infants (68%) also had Grade I ($n = 13$) or Grade II ($n = 2$) acute thymic involution.

Among the 18 infants who did not show any histological signs of shock, 14 (78 %) had positive microbial cultures, with one or more bacterial ($n = 8$), viral ($n = 1$), or both bacterial and viral ($n = 4$) isolates. *E. coli* was isolated from 4 cases among the 18 infants (22%; Table 1).

Discussion

SIDS is a diagnosis of exclusion, which means that a paucity of findings on autopsy is the rule rather than exception. This leads to difficulty in developing an animal model because there are few areas for comparison; however, an assessment of pathological evidence of shock and inflammation in tissues obtained in experiments on the rat model of sudden death and tissues obtained from the autopsies of SIDS infants found a number of striking similarities. This was most apparent between the model and infants in whom there was evidence of infection.

Evidence of stress

The use of thymic involution as a measure of pre-mortem stress offers a histological view of events that have occurred before there is any indication for concern. The grade of thymic involution has been shown to be correlated with the duration of a steroid-mediated stress prior to death in acute illness; however, this finding has not been reported as specific to any particular disease.[23] Stress that produces acute thymic involution is most often associated with severe infection or acute trauma, such as a subdural hematoma. In the absence of clinical symptoms, establishing the grade of thymic involution makes possible a reliable estimate of the duration of the stressful event prior to death.

In the rat model, thymic involution occurred more rapidly than the steroid-mediated stress reported in human fetuses and infants.[23] At 6 h and 8 h post-endotoxin challenge, the neonatal rats exhibited acute involution equivalent to 24–48 h of stress in humans. The reason for such a rapid advance is unclear. Acute thymic involution might be species-dependent; the viral infection might have primed the rats' immune systems to respond more rapidly; or the combination of viral and endotoxin challenges might have exacerbated the response of the thymus.

This timing effect can be quite variable in humans as well. In one study,[29] stress-induced changes in the thymus of humans occurred as rapidly as 3 h post-insult. The cause of differences

is unclear and has been blamed on agonal stressors; however, the animals in this study were euthanized and did not display symptoms of agonal distress prior to death. Their expression of significant thymic involution supports the hypothesis that stress may occur for several days without any outward symptoms and then be exacerbated by an infectious challenge.

In the human SIDS cases, assessing the timing between insult and death based on the histopathological findings was difficult. Using the van Baarlen criteria,[23] we calculated that 48% of the cases had been stressed for 12–24 h prior to death, while 15% had been subject to stress from 24–48 h prior to death. That few of these cases showed any significant overt clinical symptoms during the period of stress indicates that in human SIDS, as in the animal model, prior history and symptoms do not necessarily reflect the severity of the underlying physiological response.

Evidence of inflammation

Signs of inflammation and microbial colonization were found in all of the animals and many of the infants. All of the bacteria isolated from the SIDS infants were capable of increasing inflammation through components with super antigen activity and causing the inflammatory responses found in those infants. Additionally, some of the toxigenic species identified in SIDS infants have been tested in *in vitro* models in which additive or synergistic effects on the production of inflammatory mediators have been found.[30]

By far the most prevalent of organisms in the SIDS infants was *E. coli.* All of the animals had been exposed to *E. coli* endotoxin and tissues from 18 of 40 (45%) of the SIDS infants were positive for *E. coli*. Cultures positive for *E. coli* were significantly more likely to be found in infants showing histological signs of shock (14/22; 64%) than in those without such indications (4/18; 22%; $P = 0.01$). *E. coli* is a common commensal organism in humans; therefore, meticulous efforts were taken to exclude the possibility that this represented contamination. The presence of *E. coli* appears to be a predictor of shock, although other bacteria and bacterial/viral combinations might be implicated as well.

The response to multiple infectious insults is less clear. In the animal model, both a primary challenge and a secondary challenge were necessary to cause death. In humans, the implications from microbiological assessment were mixed. Of the infants, 30% had two or more micro-organisms present on autopsy. Ten of these cases exhibited a combination of bacterial and viral infections. Viral cultures are difficult to obtain post-mortem; there-fore, viral infection cannot be excluded on the basis of culture alone. There is the possibility that samples taken from post-mortem examinations carried out after significant delay might not yield all the organisms present at the time of death.[31,32] Molecular methods for detection of viral nucleic acids might provide additional evidence of mixed viral and bacterial infections and enhance their detection in these studies.

In the animal model, the timing between infectious challenges was just as important as the type of challenge. An alternative explanation in the SIDS infants may be that a combination of infectious challenges, not just viral and bacterial, are lethal. This would be more difficult to ascertain as the primary challenge in the animal model did not cause any symptoms. Perhaps an initial infection did not cause symptomatology in the SIDS infants as well.

Evidence of shock

Findings indicative of organ shock, as described by Mitchell,[14] were seen in both the animals and the SIDS infants. Liver and heart pathology was most prominent in the animal model. Heart sections displayed condensed fibers, subendocardial hemorrhages, and megakaryocytes. Liver sections showed a marbled appearance and hemorrhagic areas (Table 2). All but one

animal (17/18; 94%) exhibited this pathology, which appeared most pronounced at 6 h post-endotoxin-challenge.

Many of the shock findings were common to both the SIDS cases and the rat model. Both species died with significant symptoms of organ shock. The sub-endocardial hemorrhages in the neonatal rats appeared to be equivalent to the disseminated intravascular coagulation in the SIDS infants. Both species attempted to recruit inflammatory cells into the circulation to provide defense. In humans, megakaryocytes are abruptly released within the circulation along with other inflammatory cells, in cases of shock.[27] The presence of circulating megakaryocytes trapped in capillaries is a consistent finding on histological examination and an important indicator of shock prior to death.[28] In the SIDS cases, megakaryocytes shifted out of tic bone marrow into the circulation and other tissues; in the rat pups, megakaryocytes shifted from the spleen and over the course of 8 h increased in the circulation and liver.

The mechanism of death in the rat model appeared to be a response to shock reflected in the macroscopic and histological findings. Similarly, organ shock might have been the cause of death in many of the SIDS infants examined in this study. When there is an obvious microbiological explanation (septicemia, meningitis), the death is not classified as SIDS; however, in many of these cases association of the presence of *E, coli* or other Gram-negative bacteria with shock appeared a likely cause.

If the results obtained from the animal slides are interpreted as similar to those observed in the SIDS infants, they would not represent all cases of SIDS in this investigation, but only those in infants with increased signs of inflammation. This animal model could thus represent a subset of SIDS infants whose deaths are triggered by overwhelming inflammatory responses to what appeared to be a minor infection.

Study limitations

There are several limitations to this study including the difficulty of making comparisons between species. This is retrospective comparison with a convenience sampling of SIDS infants. There may be issue related to subject selection and the ability to control for interventions following death such as cardiac resuscitation and post-mortem change prior to autopsy. However post mortem change are very different from living changes especially when there is no further cardiac activity.

Blood cannot circulate, and there is no more diffusion within the tissues to recruit cells or make other changes. This is one way to distinguish between post-mortem and vital reactions. In the SIDS infants, the majority of the infants did not have any cardiac activity following resuscitation efforts. However, this limitation needs to be considered.

CONCLUSIONS

The conclusions from this study are descriptive in nature and meant to guide future research. In spite of their limitations, they do support several theories of sudden unexplained death in the first year of life and make the need for an animal model even more relevant. In this study, we demonstrated that the neonatal rat model mimics some of the classic findings in SIDS pathology, particularly those related to inflammation. The model permits manipulation of interactions among genetic, developmental and environmental risk factors associated with SIDS to elucidate how these might affect the responses to infectious challenges and inflammatory mechanisms implicated in these unexpected infant deaths.

SIDS, by definition, is not clearly understood. This research shows similarities between animals who died under specific controlled conditions and a sub-group of SIDS infants for

whom similar conditions were found at post-mortem. This is a reasonable animal model that has been developed for examination of the role of infection in SIDS. The pathological changes in SIDS infants are not dramatic, nor are the changes found in the animal model; however, the data observed in each instance are remarkably similar.

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Table 1

Incidence of observations/numbers of cases in each group of all SIDS infants. SIDS infants with negative histological signs of shock and SIDS infants with histological signs of shock

	All SIDS infants (*n* = 40)	**No histological shock (*n* = 18; 45%)**	**Histological shock (*n* = 22; 55%)**
Positive microbial culture	85%	78%	91%
Bacteria (1 or more)	53%	44%	59%
Virus only	8%	5%	9%
Bacteria + virus	25%	22%	27%
Total bacteria	78%	67%	86%
Infants with *E. coli*	45%	22%*	64%*

*Significant difference ($P < 0.01$) between no histological shock and positive signs of histological shock in SIDS infants with positive microbial cultures for *E. coli* by Fisher's exact test.

Table 2

Comparison of foci of shock and inflammation between the human SIDS cases and neonatal rats

	SIDS infants	Neonatal rats
Heart	Sparse hypereosinophilic myocytes with condensed and shrunken nuclei	Sparse hypereosinophilic myocytes with condensed and shrunken nuclei
		Subendocardial hemorrhages
Liver	Marbled appearance	Marbled appearance
	Fibrin thrombi in capillaries	Areas of hemorrhage and necrosis
		Increased infiltration of PMNs
		Increased megakaryocytes
Circulation	Recruitment of inflammatory cells	Recruitment of inflammatory cells
	Megakaryocytes in circulation	Increased megakaryocytes
	PMNs in the circulation	Margination of PMNs along vessel walls
	Fibrin thrombi in the capillaries: DIVC	
Kidney	Pale	Pale
	Tubular cell necrosis	Tubule degeneration
	Glomeruli retractions and exudation within the glomerular capsule	

PMNs, polymorphonuclear leukocytes; DIVC, disseminated intravascular coagulation.

Table 3

Number of animals with 0–5 histological signs of shock at times post-endotoxin administration (n = 18, total)

	Number of shock signs					
Time post-endotoxin (h)	**0**	**1**	**2**	**3**	**4**	**5**
2 (n = 5)	1	2	2			
4 (n = 5)		2		3		
6 (n = 4)				2	1	1
8 (n = 4)			3	1		

Table 4

Number of SIDS cases with 0–5 macroscopic and histological signs of shock (n = 40, total)

Number of shock signs	Total number of cases						
	0	**1**	**2**	**3**	**4**	**5**	
Histology	17	14	3	5	0	0	22/40 (55%)
Macroscopic	33	3	1	0	0	2	6/40 (15%)
Total of cases		17	4	5	0	2	28/40 (70%)