MiniReview

Animal models of sudden unexplained death

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Abstract

The etiology of sudden infant death syndrome (SIDS) is unknown but thought to be multifactorial. Several animal models have been developed that induce death without pre-existing symptoms and with pathology similar to that seen in SIDS infants; however, the relevance of these animal models to the events leading to SIDS remains elusive, in part because animal models are as varied as the potential causes of SIDS. In addition, it is difficult to find an animal model that can accurately reflect the genetic, developmental and environmental risk factors for SIDS. Comparisons between species can prove difficult but animal models provide a useful tool for evaluating potential mechanisms related to sudden unexplained death. This review focuses on models developed to examine the association of infection and inflammation with mechanisms proposed to explain sudden unexplained death.

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Keywords: SIDS; Inflammation; Immune development

1. Introduction

Sudden infant death syndrome (SIDS) is the most common cause of post-neonatal infant mortality in the developed world. The peak age of incidence is between 2 and 6 months [1,2]. It is a diagnosis of exclusion often defined as the sudden death of an infant not explained by symptoms of illness or autopsy findings. The etiology of SIDS is thought to be multifactorial and it is quite likely that many different means can cause an unexplained death leading to the diagnosis of SIDS. Proposed mechanisms of death include: respiratory and cardiac arrest secondary to re-breathing of carbon dioxide; suffocation; apnea; overheating; hypoglycaemia; vascular shock; and inappropriate inflammatory responses to infectious challenges. In many animal models, death is induced without pre-existing symptoms and with pathology similar to that seen in SIDS infants; however, the validity of animal models in relation to SIDS remains elusive, in part because animal models tested to date are as varied as the potential causes of SIDS. In addition, it is difficult to find an animal model that can accurately reflect the genetic, developmental and environmental risk factors associated with SIDS that have been identified in epidemiological studies. Risk factors, mechanisms of death and the pathological findings observed at autopsy characteristic of SIDS need to be considered in choosing an experimental system.

Although they might not reveal a definitive explanation, animal models do lend credence to certain hypotheses about the etiology of SIDS. This review focuses on models developed to examine the associations of infection and inflammation with sudden unexplained death.

2. Toxins

Siarakas et al. examined the effect of intravenous administration of six common bacterial toxins on the cardiorespiratory system in 1–3 kg rabbits: Clostridium perfringens enterotoxin and α-toxin; Staphylococcus aureus enterotoxin B; Escherichia coli heat-stable toxin [STa]; Clostridium difficile toxin A and B. They observed...
bradycardia, hypotension, and apnea with sudden death and concluded that under the right conditions bacteria could produce toxins that would cause inflammatory responses similar to those associated with endotoxin-induced shock (Table 1) [3].

Gastrointestinal pathogens are known to be one of the leading causes of morbidity and mortality in children under the age of 5 years [4]. Similarities between the gastrointestinal tract of the young rabbit and human infant provide a good model for evaluating potential effects from toxigenic bacteria [5]. A study using this model measured catecholamine responses to intravenous (IV) and intraluminal (IL) exposure in the gastrointestinal tract to these same six toxins. The study found a dose-related increase in catecholamine levels and sudden death with increasing IV doses. In contrast, intraluminal doses, even at much higher levels than those used IV, did not cause significant elevation in catecholamines. It appears that a healthy gut limits systemic toxin absorption and provides a good protective barrier. The presence of toxigenic bacteria alone is thus not sufficient to cause death in a healthy rabbit. Under certain circumstances, including combinations of viral and bacterial challenges, the investigators were able to increase toxin absorption in the infant rabbit, possibly due to disruption of the integrity of the intestinal mucosa (Table 1) [6].

Toxigenic organisms colonizing the respiratory tract best fit the common bacterial toxin hypothesis [7], and toxins of *S. aureus* have been identified in tissues of SIDS infants from five different countries [8,9]. At present, there is no animal model to assess the role of respiratory infections in SIDS, but observations on healthy human infants indicate that some of the risk factors for SIDS such as prone sleeping and respiratory virus infection increase the numbers of bacteria and the numbers of species in the upper respiratory tract of infants [10].

### 3. Models of synergistic infections

Many bacterial and viral agents have been implicated in SIDS [10,11]; however, the physiological events leading to SIDS are probably not triggered by a single bacterial or viral pathogen. There might be geographical variation in the types of infectious agents associated with these deaths. For example, the predominant staphylococcal toxins identified among Scottish SIDS infants were enterotoxins B and C; among Australian SIDS infants toxic shock syndrome toxins was predominant while among Hungarian SIDS infants enterotoxin A was most common [9]. Interactions between two or more pathogens in a susceptible host might be needed to induce a lethal response. A common objective in animal models is to develop a clearer understanding of the synergy between infectious insults and their role in stimulating an unexplained, asymptomatic death.

Lee et al. [12] demonstrated synergy in 21-day weanling rats reared in a pathogen-free environment (Table 1). Following the subcutaneous injection of nasopharyngeal bacterial isolates cultured from human SIDS infants, animals died rapidly without terminal signs of illness and with only minimal inflammatory changes in lungs, liver, or heart. Single isolates of four

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*Administration routes:* IN, intranasal; IP, intraperitoneal; IV, intravenous; IL, intraluminal; SQ, subcutaneous.
different strains of *E. coli* induced illness in one day and death in 42–48 h post-inoculation. When pathogen samples were paired (*E. coli* and *S. aureus*) mortality occurred much more abruptly, within 18 h, without preceding clinical symptoms, demonstrating the synergy between pathogens.

Although one of the defining characteristics of SIDS is an absence of obvious symptoms, more than half of SIDS infants had a mild viral infection before death [11]. In many cases, parents consulted a health care provider to report symptoms of viral illness [11]. This pattern was also observed among indigenous SIDS victims [13]. There are also data that suggest the prone position can exacerbate the consequences of a viral infection because the prone position can increase the numbers and variety of bacterial species in upper respiratory secretions [10] and also increase airway temperature, stimulating bacterial toxin production [14].

Jakeman et al. found that in a ferret model, influenza A virus in combination with different bacterial toxins caused unexplained death without clinical symptoms or postmortem histologic findings. These investigators had previously noticed 30% mortality among influenza A-infected ferrets that could have been attributed to secondary bacterial infection. They infected 1-day-old ferret kits intranasally with an attenuated influenza A (PR8) virus and allowed the animals to rest for 4 days. They then inoculated the kits with an intraperitoneal (IP) dose of one of the following toxins: *E. coli* Sta toxin or endotoxin; *Streptococcus pyogenes* streptolysins S or O; *S. aureus* α, δ, γ, or toxic shock syndrome toxin; *Corynebacterium diphtheriae* diphtheria toxin. Jakeman et al. concluded that death was due to synergy between influenza infection and bacterial toxin. They suggested that the virus could increase the ability of a toxin to enter the cell, and they also suggested that one effect could be an increase in the release of histamine and inflammatory mediators such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and platelet activating factor (PAF). Death was most likely due to central or obstructive apnea, respiratory tract spasm or anaphylaxis. For virus-infected kits, the lethality of *E. coli* endotoxin was increased 84-fold and diphtheria toxin 219-fold compared with kits treated with toxin alone. Similarities were drawn between this synergistic effect and SIDS in which interactions between virus infection and toxigenic bacteria are plausible (Table 1) [15].

A similar experiment using human peripheral blood leukocytes demonstrated a synergistic effect of influenza A and several bacterial toxins on the production of inflammatory cytokines TNF-α, IL-1β and interleukin-6 (IL-6) [16]. These models suggest that under certain circumstances, a combination of infectious insults can induce mortality characteristic of a rapid lethal septic shock. Since pro-inflammatory cytokines have been found at higher than normal levels in the body fluids and tissues of infants dying from SIDS, it is reasonable to conclude that SIDS fits within a paradigm of overwhelming inflammatory response to multiple infectious challenges.

### 4. Infectious models of apnea

Other mechanisms have been proposed to be responsible for a lethal response to infectious insults. It is known that in small infants certain infections can cause a lack of respiratory response to hypoxic events, inducing a lethal central apnea [17] or poor arousal from sleep. SIDS infants most often succumb during their sleep [18]. IL-1β, an inflammatory cytokine, is thought to cause deep sleep and apnea [19].

Several models of SIDS have examined the role of infectious challenges in relation to arousal from sleep. Identification of *Helicobacter pylori* DNA in postmortem tissues of SIDS infants suggested the following mechanism for fatal apnea as a response to gastrointestinal reflux in infants [17]. Repeated microaspiration of gastric urease produced by the bacteria might cause death due to respiratory changes related to ammonia toxicity. To examine this hypothesis, intubated adult rats were exposed to intratracheal injections of urease hourly for 3 h. Three of four rats given urease required resuscitation after the third dose, while none of the control animals given saline required resuscitation [20]. The addition of intravenous IL-1β, prolonged the urease-induced apnea [21]. There is little evidence that *H. pylori* is the primary cause of SIDS; however, this model fits with the infectious challenge theories of SIDS [22,23].

### 5. Nicotine and infection

Maternal smoking is an independent risk factor for SIDS [24]. Passive exposure to environmental tobacco smoke increases risk. Maternal smoking might contribute to the dose-dependent effect of cigarette smoke observed in some studies [25,26]. The risk of SIDS doubles with maternal smoking and is 3–4 times higher if the mother smokes more than 10 cigarettes per day [27]. It is possible that maternal smoking during pregnancy is more harmful than postnatal exposure to environmental tobacco smoke [25]. Overall, breast-feeding is considered protective for SIDS; however, this benefit is not seen with smoking mothers [26,27]. Perhaps this is due to an increase colonization with potentially pathogenic bacteria observed in smokers [28] or synergistic effects between infections and inhaled or ingested nicotine or other components of cigarette smoke by the infant [29–32].
Using combinations of the bacterial toxins in isolates from SIDS infants, one group of investigators demonstrated lethal synergy between bacterial species in a chick embryo model (Table 1) [33–35]. This model has also been used to examine the synergy between non-lethal mixtures of bacterial toxins and nicotine. The amount of nicotine in these experiments, at 0.05% of that found in one cigarette, was further diluted 32-fold but still induced a lethal effect with non-lethal doses of bacterial toxins in 42–76% of the embryos (Table 1) [36,37]. Sayers and Drucker [37] also demonstrated the potentiation of low levels of bacterial toxin by low levels of both nicotine and its primary metabolite, cotinine. Similarly, Raza et al. [38] found that cigarette smoke extract caused an elevation in TNF-α in a culture of human peripheral blood mononuclear cells previously infected with respiratory syncitial virus. Hakki et al. [39] showed that in murine splenocytes, pre-treatment with nicotine following by endotoxin increased the secretion of IL-6 and TNF-α, though concurrent treatment did not change cytokine secretion. One explanation for the lethal effect of nicotine in the experimental models is that it had a synergistic effect on inflammatory cytokine production.

6. Nicotine models of SIDS related to hypoxia

Perinatal nicotine has been shown to impair respiratory function and response to hypoxia in 0–4-day-old rats (Table 1) [40,41]. Pre-treatment with nicotine and endotoxin has been shown to interfere with autoresuscitation and produce prolonged apnea in experimental piglets [42]. Froen et al. examined apnea responses in 1-week-old piglets. The animals were intubated with intratracheal catheters then exposed to either nicotine and/or IL-1β. Fifteen minutes later they were administered 0.1 ml of acidified hypotonic saline into the subglottic space to stimulate apnea. This stimulus was repeated 5 times at 5-min intervals. The combination of nicotine and IL-1β had synergistic effects decreasing the animal’s ability to respond to apnea (Table 1) [42].

In a similar experiment, piglets were injected IV with either nicotine 20 μg kg⁻¹ or E. coli endotoxin 1 μg kg⁻¹, exposed to a hypoxic (6% O₂) environment for 30 min then reoxygenated. Mortality was observed in both the nicotine (4 of 9) and endotoxin (3 of 11) treated piglets. There were no deaths among control piglets. It is thought that either nicotine or inflammatory mediators can act as cofactors in hypoxic-ischemic neurologic injury (Table 1) [43]. This model did not examine the synergy between endotoxin and nicotine; however, it again indicates a relationship between either nicotine or inflammatory responses to infection and lethal apnea.

7. Age-dependent model of infectious challenge and synergy

These models suggest that under certain circumstances a combination of infectious challenge with or without nicotine can induce mortality. The studies considered above have not addressed the consistent observation that the peak incidence of SIDS occurs between 2 and 4 months in all countries for which accurate data are available [1]. The factors underlying increased susceptibility of this particular age range need to be examined.

There could be changes in the development of immune responses that result in a minor illness becoming a lethal event (see Gleeson and Cripps, this issue). We addressed this problem by assessing an age-dependent model of susceptibility in rats to a non-lethal strain of influenza A virus and a sub-lethal dose of endotoxin. The model produced 70–80% mortality in 12-day-old rat pups, 20% mortality in 10-day-old animals but no mortality in 16-day-old pups (Table 1) [44] (Blood-Siegfried, this issue). This narrow window of susceptibility is similar to the narrow window of susceptibility to SIDS in human infants.

In this model, a rat adapted influenza A virus (RAIV) was given intranasally. Two days later, the rats were inoculated with an intraperitoneal injection of E. coli endotoxin (0.05–0.5 mg kg⁻¹). The animals died in 6–8 h with characteristics similar to those seen in infants dying of SIDS, including liquid blood around the heart and petechiae in the respiratory tract. Increasing the endotoxin dose above 0.2 mg kg⁻¹ did not significantly increase mortality but did increase morbidity. Mortality was most significant between 10 and 16 days of age; adult rats did not succumb to the dual infectious challenge. Our data clearly identify age as a key risk factor for mortality.

We have suggested that in the developing immune system, influenza A virus changes the kinetics of response to a second infectious challenge such as endotoxin, and the regulation of inflammatory cytokines, acute phase proteins and reactive nitrogen species that normally promote a protective response. The developing immune system is either unable to tolerate these changes and/or unable to dampen the response resulting in death [44] (Blood-Siegfried, this issue). A dramatic drop in night-time cortisol levels occurs in human infants between 2 and 4 months of age due to the normal developmental changes in circadian rhythm [45,46]. Adults have higher inflammatory responses at night during periods that correspond with low blood cortisol levels [47]. The dramatic drop in night-time cortisol levels associated with development of circadian rhythm might explain why infants in this age range are more vulnerable to an exaggerated inflammatory response and why most SIDS deaths occur at night [48].
8. Animal and human development of inflammatory responses to infectious agents

Animal models have been important for developing and testing ideas about the physiological events leading to SIDS; however, it is difficult to show a direct connection to human immune development. The murine model is often used to examine immune development [49]. Mammals with gestations longer than 60 days are more immunologically mature at birth than animals with shorter gestations; however, the rat and mouse, with 21-day gestations each, mature faster after birth than the human infant. They both have similar development: weaning occurs around 21 days, and they are considered adult by 8–10 weeks [50]. Although this is still a matter of opinion, most researchers consider the immune system in the human neonate (0–4 weeks) approximates the first 7 days of life in the mouse [51–53] and rat [54].

The ability to produce pro-inflammatory cytokines is normal at a very early age [55–58]. Rats have the ability to produce inflammatory cytokines in response to endotoxin and other insults prenatally [59] and in the first day of life in response to hypoxia [60]. Inflammatory cytokines are secreted by infant peripheral blood mononuclear cells and cord blood monocyte-derived macrophages in response to bacteria and viruses [61,62].

The numbers of B cells in the lymph nodes of the rat increase during gestation and at birth contain both IgM and IgG secreting cells. Immunoglobulin is transferred across the placenta, and IgG is found in concentrations similar to those in term human infants at birth [63]. Immunoglobulins are also absorbed in the GI tract from colostrum and breast milk, providing immunity in the first 21 days of life [64]. Adult levels of IgG and IgM are not fully developed until 2–3 months in the rat [50] and 2 years in humans [65]. In both rat and human, the neonatal B cell is a poor antigen-presenting cell, which can limit T-cell responsiveness [66].

Immunizations stimulate antibody and provide a good reference point for comparing systems. Tolerance to an antigen is often the result of immunization at a very young age. Tolerance to sheep red blood cells (SRBCs) is induced in 1-day-old rats, and partial tolerance is induced in 3-day-old rats, with a significant increase in plaque forming cells at 11–21 days of life. By 34 days there is an adult response [50]. Most importantly, rat pups are able to respond to heterologous erythrocytes starting around 10 days of age and are able to make antibodies to SRBC. Rat pups 10–20 days of age respond to antigenic stimuli and produce an IgM response at about 40% of the adult level [67]. Tolerance can also be induced in human infants when immunized at birth with certain antigens. Diphtheria immunization will stimulate tolerance at birth and begin to provide a strong antitoxin response between 2 and 6 months of age [68].

In spite of differences in timing, the kinetics and overall sequence of events in T-cell development are similar in humans and rodents [69]. They both develop a competent antigen-specific cytotoxic lymphocyte response during the newborn period. Japanese B encephalitis virus is fatal in 7–8-day-old rat pups, but by 12 days of age only a few are susceptible and by 21 days there are no apparent symptoms [50]. In human newborns, the response to viral infections is immature; however, by 2 months of age memory T cells are fully capable of response [70]. There is delayed acquisition to antigen specific cell mediated immunity [71]. Newborn humans are anergic to skin testing, and there is a delay in a cutaneous response in the form of delayed type hypersensitivity for the first month of life, this decreased responsiveness may last up to 3–4 months of age [65,72]. Cellular immune responses to viral antigens are delayed in mice, rats and humans.

Certain cytokines, interferon gamma (IFN-γ), and interleukin-4 (IL-4) are not produced very well by the naive T helper, CD4 type cells in the neonate [70,73]. These particular cytokines have an important role in the immune response. Their decrease might be an important cause of normal immunodeficiency found in the neonate [70]. This deficiency delays antigen specific immunity, and selected immunoglobulin production [70,73]. Mouse lymphocytes proliferate in response to Con A and pokeweed mitogen in early neonatal life; however, adult responses are not seen until 2–3 weeks of age [50]. Newborn murine spleen cells proliferate poorly to Con A in the first 2 weeks of life [74]. Data in our lab support this lack of proliferative response of rat spleen cells to Con A and pokeweed mitogen before 10 days of age, with proliferation still below adult levels at 17 days of life (data not shown). The ability of T cells to proliferate in response to a mitogen is similar in humans, mice and rats.

In early life all three species have areas of immunity that are less responsive than the adult [75,76]. Immunologic competence is achieved in the first few weeks of life in laboratory rodents. Although the murine model defines much of what is known about immune development, we think that rat immune development also provides a useful model for human infants. The rapid development of the immune system in the rat makes each day a significant time point in comparison to human infants. The response to antigens varies depending on the antigen, the dose of antigen, the mode of presentation and the animal species used, accounting for the minor differences observed in the response [69]. The peak age for SIDS in humans, between 2 and 4 months of age, is a time of low levels of protective antibody due to loss of passively acquired maternal antibody and early response to bacterial toxins and viral antigens. This corresponds to a similar immune point in the rat,
between 10 and 20 days of life, also a time of early antigen response.

9. Summary

The physiological events leading to SIDS are thought to be triggered by a number of pathogens and inflammatory responses that affect the function of many organ systems, including the immune system. Animal models provide a useful tool for evaluating potential mechanisms related to sudden unexplained death. Comparisons between species are difficult, but some characteristics of immune development are clearly sequentially related across species. Our model offers a unique way to evaluate the role of immune development and its relationship to potential pathogens in the etiology of SIDS.

References


