HYPOTHESIS

Effects of nicotine on bacterial toxins associated with cot death

N M Sayers, D B Drucker, D R Telford, J A Morris

Abstract
Toxins produced by staphylococci and enterobacteria isolated from the nasopharynx of cases of sudden infant death syndrome (SIDS) have a lethal effect when injected into chick embryos. If the toxins are progressively diluted the lethal effect disappears, but certain combinations of toxins show synergy so that if sublethal doses are mixed a highly lethal effect is produced. In this paper it is shown that nicotine at very low concentrations (less than that produced in man by 0.05 cigarettes) potentiates the lethal action of certain SIDS associated bacterial toxins and markedly potentiates the lethal action of synergistic combinations of bacterial toxins. These results could explain, at least in part, why parental smoking increases the risk of SIDS. They also provide further support for the common bacterial toxin hypothesis of cot death.

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Infants who are put to sleep on their backs, who are not overwrapped, and are not exposed to tobacco smoke are at decreased risk of sudden infant death syndrome (SIDS).1-4 This information has been put to good use in national publicity campaigns which have been associated with a fall in the number of SIDS cases but the precise way in which these factors interact with the pathogenetic mechanism of SIDS is not known. In recent years, however, one theory which can explain the main epidemiological features of SIDS has gradually been gaining ground.5,6 The idea is that sudden death is due to toxins produced by nasopharyngeal bacterial overgrowth that occurs after a viral upper respiratory tract infection. The theory directly predicts the age distribution of SIDS,7 the winter excess of cases,8 and the association with the prone sleeping position.7 It has also led to a number of laboratory based studies with promising results. In particular work has shown that SIDS cases are more likely than age matched healthy infants to harbour bacteria in their nasopharynges which produce lethal toxins as assessed by a chick embryo bioassay.8 Furthermore when tested in the same system certain combinations of toxins from the same infant show synergy with the result that a lethal effect is produced by very low toxin concentrations.9

In this paper we present evidence that nicotine interacts with selected bacterial toxins potentiating the lethal effect of both single toxins and synergistic combinations. This suggests one possible explanation for the role of tobacco smoke in SIDS.

Methods
Bacterial isolates from the nasopharynx of SIDS cases had been obtained previously and stored at −70°C.10 To prepare toxins the bacteria were grown on a semipermeable membrane overlaying a defined agar.8 In this system nutrients from the agar diffuse through the membrane to support surface growth but toxins secreted by the bacteria over a molecular weight of 12 000 are retained on the surface. The growth was then washed from the membrane and centrifuged and filtered to separate toxin secretions from live organisms. The crude toxin preparations were subsequently diluted to a standard optical density and tested for lethality by injection into the chorioallantoic vein of 11 day old embryonated chick eggs. Finally the eggs were incubated and tested for viability after 18 hours.

In these experiments a Staphylococcus aureus and Klebsiella pneumoniae isolated from one case of SIDS and S aureus and Escherichia coli isolated from a second case of SIDS were used. These pairs had been shown to cause lethal synergy in previous experiments. A series of doubling dilutions were prepared for each toxin preparation. Eleven eggs were injected with each dilution and the percentage lethality calculated. In each case a dilution was chosen which gave a low percentage kill; this was the neat concentration used in subsequent experiments.

A solution of nicotine was also tested in the eggs in the same way as for the toxin preparations. A range of concentrations from 1 mg/ml to 7.8 µg/ml was tested. A concentration of 800 ng/ml (equivalent to 40 ng/egg) was found to produce a lethal response in 22% of embryos. This was used as the neat solution of nicotine.
A series of experiments were then performed in which doubling dilutions of the neat solutions (neat, 1/2, 1/4, 1/8, 1/16, 1/32) were tested separately and in combination in chick embryos. Each test involved injecting 11 to 15 eggs and calculating percentage lethality. Occasionally injections led to bleeding and these eggs were replaced. The total volume of injection for each chick embryo was held constant in these experiments at 50 μl.

### Results

The results of the two series of experiments are shown in the table. The first pair of organisms (S. aureus (A) and K. pneumoniae (B) come from one case and the second pair (S. aureus (D) and E. coli (E)) come from a second case. The individual bacterial toxins were non-lethal in this series of experiments but when combined they showed a synergistic effect – that is a lethal response that was greater than expected from simple addition. Furthermore nicotine showed a synergistic effect with individual bacterial toxins. The most marked effect, however, occurred when nicotine was added to the bacterial toxin combinations. At dilutions beyond 1/16 when individual solution had no effect the combination produced lethal rates between 46% and 82% (p<0.00001).

### Discussion

Many theories have been advanced to explain SIDS but few are consistent with the full range of epidemiological features and they rarely lead to ideas that can be tested in the laboratory. The common bacterial toxin hypothesis, however, is an exception. This theory directly predicts the precise form of the age distribution curve of SIDS, which is its most consistent and characteristic feature. It also explains the seasonal incidence and the association with the prone sleeping position. The theory has proved amenable to laboratory investigation. The nasopharyngeal bacterial flora of SIDS cases is abnormal when compared with age matched healthy infants. There is increased carriage of staphylococci, streptococci, and enterobacteria. Furthermore toxins produced by the SIDS bacteria are more likely to have a lethal effect in the chick embryo than are toxins from the bacteria of the age matched comparison group. Certain combinations of toxins, particularly from staphylococci and enterobacteria, obtained from SIDS cases show lethal synergy in the chick embryo. Lethal synergy has also been demonstrated between the same organisms in gnotobiotic weanling rats and between bacterial toxins and influenza virus in ferrets. Immuno-histological studies have shown staphylococcal exotoxins in the kidneys of SIDS cases and there is a deficit of antibody to endotoxin core in SIDS cases suggesting episodes of endotoxaemia before death.

Parental smoking increases the risk of SIDS. This could be due to direct action on the infant in utero producing growth retardation which is independently associated with SIDS. Another possibility is that tobacco smoke could act as an irritant on the upper airways of the infant after birth, impairing mucociliary clearance and increasing the risk of upper respiratory tract infection. The work reported here, however, suggests a third possibility which is that nicotine increases the risk of sudden death in infants exposed to synergistic combinations of bacterial toxins at the time when they lack specific antitoxin antibody. An adult absorbs approximately 1 mg of nicotine from one cigarette. The equivalent dose for a chick embryo, which is over 1000 times smaller, is 750 ng. In these experiments eggs injected with the neat solution of nicotine received 40 ng which would produce a nicotine concentration in the embryo of the same order as that produced in man by 0-05 cigarettes. But even when diluted a further 32-fold the nicotine solution was able to transform a non-lethal mixture of bacterial toxins into one which killed 42% and 76% of the chick embryos. There are obvious limitations in extrapolating results from chick embryos to human infants but the experiments reported here do point to a possible role for nicotine levels, which can be achieved by passive smoking, in potentiating bacterial toxin induced infant death.

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9. Drucker DB, Alhyi HS, Morris JA, Telford DR, Gibbs A. Lethal synergistic action of toxins of bacteria isolated from
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