Simian virus 40 and mesothelioma in Great Britain

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Background  Simian virus 40 (SV40) is a DNA virus that has been shown capable of infecting and transforming cells in various species. Laboratory studies have suggested that inoculation with SV40 is associated with various types of cancer, including mesothelioma.

Aims  To test the hypothesis, via an ecological analysis, that exposure to SV40 via contaminated polio vaccines is a risk factor for mesothelioma in humans.

Methods  Mesothelioma mortality rates in Great Britain for two birth cohorts likely to have been exposed to SV40 via poliovirus vaccination were compared with a birth cohort likely to be largely unexposed.

Results  There was some evidence for both males (P < 0.05) and females (P < 0.05) that the mesothelioma mortality rates were higher in the first exposed cohort: rate ratio (RR) = 2.4 [95% CI (confidence interval) 1.2–5.0] and RR = 3.7 (95% CI 1.0–14). However, in the second exposed cohort, mortality rates were elevated in females only, and the evidence was slightly less convincing (P = 0.055).

Conclusion  Although the results for females show a reduction in the mesothelioma mortality rate coinciding with the introduction of the SV40-free Sabin polio vaccine, the absence of a similar result in males and of a priori biological evidence of a sex-specific SV40 effect, makes chance the most plausible interpretation of these findings.

Key words  Great Britain; mesothelioma; polio vaccine; simian virus 40.

Introduction

A causal link between occupational asbestos exposures and the occurrence of mesothelioma in humans has been established for many years [1]. Recently published updated mesothelioma statistics for Great Britain by geographical area and occupational group [2] and projections of the future burden of mesothelioma mortality in Great Britain [3] are consistent with the majority of mesothelioma cases being caused by asbestos exposure. Evidence for other risk factors for mesothelioma is more limited.

One potential risk factor that has been studied is infection by the DNA virus simian virus 40 (SV40) which was found to contaminate some polio vaccines administered in the late 1950s and early 1960s. SV40 is capable of infecting and transforming cells from various species [4]. In 1961 it was identified in the kidney cells of rhesus monkeys used from the early 1950s to culture poliovirus for the Salk polio vaccine [5]. Consequently between 1955 and 1963, millions of people in the western world were likely to have been exposed to live SV40-contaminated vaccines [6–9]. Following its discovery, SV40 became the object of some intense investigation to examine whether or not it posed a risk to health.

Epidemiological studies making comparisons of cancer incidence rates in cohorts with and without exposure to SV40-contaminated vaccine have not provided any clear evidence for an association between SV40 and mesothelioma in humans [10–14]. These studies have suffered from a lack of statistical power due to the relatively short latency period for mesothelioma. The authors of these studies recommended further research and monitoring of the issue.

The annual reports of the Ministry of Health for the years 1955–67 gave information about polio vaccination in Great Britain during the period when Salk vaccines were administered [15]. In January 1956, local authorities within Great Britain were invited to participate in a scheme to make available courses of Salk polio vaccine to all children born between the years 1947 and 1954 inclusive. A full course of vaccination consisted of three separate injections with the possibility of a further booster injection, although not all the courses of vaccination were
completed. At the time, 144 of 146 local authorities in England and Wales agreed to take part. The coverage of the vaccine within these local authorities gradually increased and, by autumn 1958, the vaccine was available on request to all persons born since 1933—including expectant mothers. In February 1960, the coverage was extended and included all persons under the age of 40, although the main emphasis of the programme remained the vaccination of children, most of whom were given the course in their second year of life.

In response to the detection and isolation of the SV40 contaminant within the Salk vaccine, steps were taken to eliminate it from future vaccines [16]. As a result, in 1962 a new oral form of poliovirus vaccine (Sabin), which was free of SV40 contamination, was made available for routine vaccination. This new vaccine was used for 78% of inoculations in 1962, 96% in 1963, and by 1967 this proportion had increased to ~100%.

Estimates of the proportion of individual doses of Salk vaccine that actually contained live SV40 vary. Shah and Nathanson [7] estimated the contamination rate to be between 10 and 30%. However, because most persons received three or four vaccination injections of the Salk vaccine over a period of time, the probability that at least one of these vaccines contained live SV40 was quite high. In addition, the level of SV40 contamination within doses would have varied quite considerably.

The aim of the present study was to investigate the hypothesis that infection by SV40 via contaminated polio vaccines has increased mesothelioma risk by comparing mesothelioma death rates among appropriate birth cohorts within Great Britain. The relatively high mesothelioma mortality rates in Great Britain [2], and the longer follow-up period now available compared with previous studies, gave increased statistical power to detect any potential effect.

Methods

Estimated percentages of persons born in each year from 1943 to 1965 who were inoculated with potentially SV40-contaminated Salk polio vaccine were extracted from the annual reports of the Ministry of Health for the years 1955–67 [15]. These percentages were used to inform the choice of appropriate birth cohorts for which mesothelioma mortality rates would be compared. The cohorts were chosen in order to give as low as possible a proportion of SV40-exposed persons in the cohort defined as unexposed and as high a proportion as possible in the cohort defined as exposed. However, the need to maximize the amount of available follow-up and to choose cohorts as close together in time as possible (in order to limit any differences in the levels of population asbestos exposure, which may have changed over time) was also taken into account.

Mesothelioma death rates for the cohorts were based on the data from the British mesothelioma register—a source of mesothelioma mortality data derived from textual descriptions of death certificates rather than a reliance on cause of death coding [2]. The mesothelioma register received approval of the Department of Health’s Patient Information Advisory Group (PIAG) in accordance with the Health and Social Care Act 2001 in December 2003. Since then it has been subject to annual review by PIAG.

Cohort comparisons were made within sex with age restricted to allow equal follow-up for all those born within the cohorts of interest—that is, those aged 38 years or less at death. In the calculation of mortality rates, the total person-years at risk for each cohort was calculated by summing population data over time using annual national population estimates for the period 1968–2004 by sex and age. Age-standardized incidence rates were calculated according to the age structure in the unexposed cohort using 5-year age groups (apart from the lowest age group which consisted of 7 years). Rate ratios (RRs) and confidence intervals (CIs) were then calculated.

Results

Figure 1 shows the estimated proportion of persons born in each year from 1957 to 1966 who were inoculated with potentially SV40-contaminated Salk polio vaccine in Great Britain. Inoculation rates fell dramatically in 1961 to 21% and fell again in the following year.

The unexposed cohort was chosen to be those born in 1962–66. An estimated 4% exposure level in 1962 was judged to be low enough for inclusion in the unexposed cohort when balanced against the reduction in follow-up time resulting from choosing a later start date. It was considered difficult to choose an exposed cohort that optimally balanced the requirement to have the maximum exposure level possible and the need to choose cohorts as close together in time as possible to minimize the effect of
changes in exposure to asbestos as a confounder. Therefore, two comparisons using two different exposed birth cohorts were carried out. The first (1951–55) was chosen to maximize the proportion of persons who were exposed to SV40, and the second (1956–60) was chosen to be as close in time as possible to the unexposed cohort. The comparisons included all deaths among persons aged 17–38.

Table 1 shows that for both males and females, there was some evidence ($P < 0.05$) that the mesothelioma mortality rate was higher in the potentially exposed group (the 1951–55 birth cohort) than the unlikely exposed group (the 1962–66 birth cohort): RR = 2.4 (95% CI 1.2–5.0) and RR = 3.8 (95% CI 1.0–14), respectively, for males and females.

In males, there was no difference in the mortality rate between the other potentially exposed group (the 1956–60 birth cohort) and unlikely exposed groups: RR = 0.93 (95% CI 0.39–2.2). However, in females the mortality rate was higher in this exposed group, although the evidence is not as strong ($P = 0.055$): RR = 3.5 (95% CI 0.93–13).

**Discussion**

In our analysis we found that in females, both potentially SV40-exposed birth cohorts had a higher mesothelioma rate than the birth cohort unlikely to be exposed; however, in males, the rate was higher only in the earlier potentially SV40-exposed birth cohort. These results are somewhat difficult to interpret. They could be consistent with there being no effect due to SV40, in which case the lack of a further reduction in the male mesothelioma rate beyond 1960 would suggest that reductions in asbestos exposure for men had levelled off. This would suggest some continuing occupational exposure in men beyond 1980. Conversely, the results could be consistent with there being an effect due to SV40 but that it is being masked among males by an increase in population asbestos exposure at younger ages. Finally, the results may suggest that SV40 has a causal association in the aetiology of mesothelioma among females, but not among males; however, there is currently no biological evidence to support this. Any changes in the mesothelioma rate that were due to a genuine SV40 effect acting within a specific cohort could be quite marked, whereas the effects of changes in asbestos exposure are likely to be more gradual. Given the small numbers of deaths in our analysis, it is not possible to judge whether the observation in females amounts to a real step change or part of a more gradual effect.

Various investigators have conducted studies to address the possible association of SV40 with human malignancies and SV40 has been detected in various human tumours including pleural mesotheliomas [17–19]. A panel of experts at an international consensus meeting held at the University of Chicago in April 2001 concluded that there is overwhelming evidence that SV40 is capable of infecting humans and that it may be involved in the pathogenesis of some human mesotheliomas, though the mechanisms were acknowledged as being largely not well understood [20]. A recent review concluded that the evidence for a causal link between SV40 and the development of human tumours is most convincing for the mesothelioma tumour type [21]. However, set against this evidence are the findings of a recent study which concluded that SV40 is at most only rarely present in human mesotheliomas and that its positive detection in many polymerase chain reaction-based studies may result from the wide use of assay designs susceptible to false-positive results [22]. Most human epidemiological studies have not found a positive association between SV40 and increased mesothelioma risk [11, 13, 14], though one study did suggest there may be an association [12]. A further study suggested that SV40-exposed cohorts had not yet reached an age at which any increased risk might be detected [10]. Reviews have generally concluded that there was inadequate evidence to conclude whether or not the contaminated polio vaccine caused cancer [16].

Our analysis was limited for a number of reasons. Firstly, the long latency period of mesothelioma coupled

**Table 1.** Results of comparison of mesothelioma mortality in two potentially SV40-exposed birth cohorts with an unexposed birth cohort

<table>
<thead>
<tr>
<th>Sex</th>
<th>Birth cohort</th>
<th>SV40 exposure</th>
<th>Mesothelioma mortality</th>
<th>Rate ratio (relative to 1962–66)</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Deaths</td>
<td>Age-standardized rate per million</td>
<td>Lower limit</td>
</tr>
<tr>
<td>Males</td>
<td>1951–55</td>
<td>Potentially exposed</td>
<td>22</td>
<td>0.54</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>1956–60</td>
<td>Unlikely</td>
<td>9</td>
<td>0.20</td>
<td>0.93</td>
</tr>
<tr>
<td>Female</td>
<td>1951–55</td>
<td>Potentially exposed</td>
<td>9</td>
<td>0.22</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>1956–60</td>
<td>Unlikely</td>
<td>3</td>
<td>0.060</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>1962–66</td>
<td>Unlikely</td>
<td>3</td>
<td>0.060</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*Age-standardization carried out on basis of person-years in age groups 17–23, 24–28, 29–33 and 34–38 in the unlikely exposed cohort (1962–66).*
with the fact that most exposure to asbestos is occupational [23], meant that, as in previous studies, the age range in this study only consisted of a very small subgroup of all mesothelioma deaths. The small number of cases affects the statistical power of the analysis and the further passage of time will allow an increase in the number of cases that might be exploited in epidemiological studies. The subgroup of mesothelioma deaths at young ages may not be a representative sample of all mesothelioma deaths. Persons this young are likely to either have had childhood exposure to asbestos or occupational exposure very early in their working lives, in which case they would represent those cases with the shortest latencies. The small number of cases also prevented a full analysis of mesothelioma subtypes (pleural or peritoneal). However, restricting the cohort comparisons to pleural mesotheliomas showed the same pattern of reductions in rates as those for all mesothelioma types.

A further limitation relates to the use of Salk vaccination rates as a proxy for the rate of SV40 infection. The levels of SV40 exposure vary considerably among individuals inoculated with contaminated polio vaccine and, because no adequate data source exists to measure this, the study can be considered to measure the average population level exposure from inoculation with potentially SV40-contaminated Salk polio vaccine. Although this may have a diluting effect on the results, this should not cause any bias in the results because there is no evidence of a systematic difference in the SV40 contamination rate of polio vaccines or in the number of injections actually administered between cohorts. Our study assumes there is a negligible prevalence of SV40 infection among persons born between the years 1962 and 1966. This is based on our low estimate of the proportion of persons vaccinated with potentially SV40-contaminated Salk polio vaccine. However, there is evidence to suggest that SV40 infection can occur in humans by other means. Butel and Lednicky [24] observed that a substantial proportion of individuals with no risk of exposure to SV40 through contaminated polio vaccines (∼10%) had SV40-neutralizing antibody and suggested that this indicates an alternative source of human infection by SV40. Fisher et al. [12] reported the ability of the SV40 to replicate, generate a subclinical infection and spread through oral and respiratory routes. Thus, SV40 may be more widespread in the general population than is defined by our assumption that potentially contaminated polio vaccines are the sole source of infection. This would have a diluting effect on the results, which would reduce the power of the study to detect an association between SV40 exposure and the subsequent contraction of mesothelioma, since there would be an increased proportion of persons in our defined unexposed cohort who have actually been infected with SV40.

Overall, our interpretation of the difference in the findings for males and females is that it is due to chance and that our study does not provide strong evidence to support the hypothesis that SV40 is a risk factor for mesothelioma.

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References
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