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The Prevalence of Chromosomal Aberrations in Argentine Air Crew Members

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ABSTRACT. The authors analyzed the effects of chronic exposure of Argentine air crew members to low doses of ionizing radiations. Genetic damage induced by either low doses or low rates of ionizing radiation was higher than expected. Seventy-one heparinized blood samples were obtained from technical ground workers (group A; *n* = 10), pilots of domestic flights (group B; *n* = 14), pilots of transequatorial flights (group C; *n* = 17;), pilots of transpolar flights (group D; *n* = 17) and retired pilots (group E; *n* = 10) The frequency of dicentric chromosomes was higher in groups B and C compared with groups D and E. These observations suggest that the exposure of the aircraft to ionizing radiations may induce chromosomal aberrations. However, dicentric chromosomes in both domestic and retired pilots are still high compared with dicentric control participants.

KEYWORDS: air crew, chromosomal aberrations, cosmic radiation

Studies of the biological effects of ionizing radiation have mainly focused on the consequences of single exposures to relatively high doses. Several reports on the analysis of chromosomal aberrations in peripheral blood lymphocytes after acute accidental exposures could be mentioned as examples.[1–5] In contrast, there is little information available about the biological effects of low-level exposure, even though these effects are significant in a large number of populations. Experimental results obtained during the past decade indicate that genetic damage induced by either low doses or low rates of ionizing radiation is more prevalent than expected.[6–12]

Human cytogenetic biomonitoring studies have been proposed as a tool to assess the possible genotoxic effects of nonintentional exposure. It is possible that the most conspicuous example of chronic exposure to low doses of ionizing radiations appears in the air crew members of commercial transcontinental flights. Radiation comes from the sun and galaxy, strikes the upper atmosphere, and produces a spectrum of charged particles that are potentially harmful for human beings. At the flight altitudes of civil aircraft, the radiation field is highly modified owing to the transport of primary cosmic particles, both galactic and solar, through the earth's atmosphere. On average, galactic cosmic radiation makes up the majority of on-board exposures, representing more than 90% of radiation, even in a supersonic aircraft. The on-board field is composed predominantly of secondary particles. At flight altitudes between 9 and 18 km, roughly one-half of the dose equivalent is delivered by neutrons (including the high energy component) and the second half by particles with low linear energy transfer (LET), mostly electrons and protons.[13–14] Cosmic radiation (a natural radiation that originates outside the Earth) increases with altitude, background radiation from radioac-

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tive substances on earth decreases. The cosmic radiation dose received by passengers and crew of aircraft or spacecraft in flight does not increase linearly with altitude. Current commercial aircraft normally fly at altitudes between 6 and 12 km (where levels of cosmic radiation are 0.4–0.44 μSv/h and 2.55–2.78 μSv/h, respectively). The Concorde that achieved height of 16 to 18 km received a dose between 3.77 and 4.09 μSv/h.

Radiation levels increase with latitude; that is, lower in the vicinity of the equator than in the North and South Poles. At commercial flight altitudes the radiation exposure comes directly from neutrons, electrons, and photons, with much less direct contributions from cosmic rays (see Table 1). The radiation dose also increases with altitude, doubling approximately every 6,500 ft. The dose also increases with latitude. For example, north of 50º N, levels of radiation are about twice as much as levels on the equator.[17–20]

Kelly et al[15] recently noted a severe lack of biological data of the effects of cosmic radiation, especially of neutrons on humans. More recently, the neutron and gamma dose equivalents for 2 transpacific flights of 73 hours total duration were found to be 39.7 and 74.0 μSv, respectively.[16]

Over the last few decades, exposure to ionizing radiation from galactic and solar sources has been recognized as a potential cancer risk for aircraft crews. Because of this cosmic radiation, the dose equivalent rate is estimated to be about 6 μSv/h[21] at current air travel altitudes of 9,000–12,000 m (30,000–40,000 ft). This dose equivalent rate roughly doubles at an altitude of 18,000 m (60,000 ft), and may even reach levels of 0.5 to 1.0 μSv/h during relatively brief periods of so-called solar flares.[21] Given the variety in flying times and routes, annual dose equivalents received by flight personnel may range from 0.2 to 9.1 μSv.[19] Even in the worst case, it is not likely that the dose equivalents of air crew members exceed the National Council on Radiation Protection and Measurements recommendation for occupationally exposed workers of 20 μSv/y averaged over 5 years.[17,22] Nevertheless, the dose equivalents do exceed the recommended level for exposure to ionizing radiation in the general public, which is 1 μSv/y. Elevated frequencies of chromosome aberrations or DNA damage observed in civil pilots were closely correlated to the continuous exposure to low doses of ionizing radiation.[23–26] However, contradictory results were reported in another study carried out with cabin attendants.[26]

Table 1.— Components of Cosmic Radiation at Heights of Normal Flight

Particle	%
Neutrons	33–52
Proton	1–28
Electrons and photons	17–41
Muones	2–11
Charged piones	< 1

When the relationship between flight hours and the frequency of chromosomal aberrations was estimated, a linear pattern was found for the following categories: < 11,350, 11,350–15,000, 15,000–17,000. But in the fourth group (> 17,000), the risk decreased and was not significantly higher than in controls.[28] On the other hand, epidemiological cancer studies among aircraft crews reported excesses for some types of malignancies.[29–31]

In Argentina, all international flights are long distance, either transequatorial or transpolar. International Argentine crews are thus exposed to higher levels of ionizing radiation than crews on domestic flights. For instance, the weekly transpolar Buenos Aires–Auckland flight spends between 12 to 14 hours at a cruising altitude of around 40,000 ft. Epidemiological in vivo and in vitro studies may give valuable information about the degree and the extent of exposure, as well as the differences in individual susceptibility. Moreover, data obtained from these studies could contribute to genetic risk estimation.

Taking into account the latitude and altitude risks, the exposure of members of Argentine crews may vary substantially according to the type of flights conducted. The aim of this work was to evaluate the frequency of chromosomal aberrations in crew members of Argentine flights of domestic, transequatorial, and transpolar routes. Additionally, we estimated the frequency of aberrations in retired pilots in order to evaluate the persistence of this damage through time.

METHODS

Experimental Procedure

Seventy-one heparinized blood samples were obtained from technical ground workers (group A; *n* = 10), pilots of domestic flights (group B; *n* = 14), pilots of transequatorial flights (group C; *n* = 17), pilots of transpolar flights (group D; *n* = 17), and retired pilots (group E; *n* = 10). Pilots of domestic flights had no international flight hours. International pilots had been employed as pilots for the past 10–24 years. Prior to blood collection, each individual completed a structural questionnaire specifying sex, age, smoking habits, alcohol consumption, dietary habits, previous exposure to diagnostic X-rays, use of therapeutic drugs, and work-related exposure to hazardous agents. None of the subjects had elevated alcohol consumption or a deficient diet, smoked, or had received X-rays, genotoxic agent exposure, or therapeutic drugs during the past year. Therefore, we considered only age and flight hours as possible confounding factors. We provided all participants with specific written information about the aim of the study. All participants gave their written informed consent. Table 2 shows the average age, the standard deviation, and maximum and minimum age of the air crew members in the different groups. Table 3 shows the averages of total flight hours and total international flight hours.

Table 2.— Age and Number of Exposed and Control Subjects

Group	Total	*M*	*SD*	Age range (y)
A	10	37.2	6.0	48–31
B	14	37.1	8.4	57–27
C	20	46.2	8.6	57–31
D	17	50.6	5.8	59–40
E	10	65.2	7.0	74–52

Note. Group A = controls; group B = pilots of domestic flights; group C = pilots of transequatorial flights; group D = pilots of transpolar flights; group E = retired pilots.

Table 3.— Total of Flight Hours in the Different Studied Groups

	Total flight hours		Total international flight hours	
Group	*M*	*SD*	*M*	*SD*
B	6,947	4,150	0	0
C	10,715	4,213	6,779	2,805
D	12,200	3,863	5,876	4,474
E	22,070	1,704	12,950	4,064

Note. Group B = pilots of domestic flights; group C = pilots of transequatorial flights; group D = pilots of transpolar flights; group E = retired pilots.

Cytogenetic Analysis

Five mL of blood samples were collected in Vacutainer tubes. Blood samples were cultured for 52 hours in Ham’s F10 medium, which was supplemented with phytohemaglutinin at the concentration recommended by the supplier: penicillin (60 IU/mL), streptomycin (50 μg/mL), and 10% fetal bovine serum v/v. Two hours before harvesting, colchicine (1 μg/mL) was added to the medium. After centrifugation, lymphocytes were resuspended in 5 mL of 0.075 M KCl and incubated at 37ºC for 40 minutes. Fixation was carried out with methanol/acetic acid (3:1) at room temperature. Chromosomal preparations were made by dropping the cell suspension onto a slide and then were stained with 5% Giemsa solution. Chromosomal preparations were blind counted and scored for aberrations blind on coded slides. A total of 200 metaphases per donor were scored to check the incidence of structural chromosomal aberrations.

Statistical Analysis

To evaluate whether differences among chromosomal aberrations of all types among different groups were significant and caused by the exposure to cosmic radiation, we used Poisson regression analysis. In this way, we analyzed the possible influence of confounding factors such as age and flight hours (see Experimental Procedure section). For dependent variables, we considered both the control group (A) and the different pilot groups (B to E). The independent variables were age and flight hours.

RESULTS

The cytogenetic analysis of the 71 blood samples showed monochromatid (a fracture in 1 chromatid generating an acentric fragment) and isochromatid (simultaneous fracture of both chromatids) breaks, as well as dicentric chromosomes. Chromosomal aberrations were presented as the cumulative number of aberrations per 100 metaphases (see Table 4). Taking all types of aberrations (abnormal metaphases) together, we found significant differences when transequatorial (group C; $p < .03$), transpolar (group D; $p < .01$), and retired pilots (group E; $p < .01$) were compared with domestic pilots and control participants (groups A and B, respectively; $p < .03$; see Table 4 and Figure 1). No differences were found among groups C, D, and E or between groups A and B. The multivariate analyses also showed that confounding factors (see Materials and Methods section)

Table 4.— Incidence of Chromosome Aberrations per 100 Cells

	Abnormal metaphases		B’		B’’		Dicentrics	
Group	*M*	CI	*M*	CI	*M*	CI	*M*	CI
A	5.5	4.2–7.2	1.6	0.8–2.6	3.8	2.6–5.2	0.0	0.0–0.4
B	3.5	2.6–4.8	1.7	1.2–2.6	0.07	0.0–0.2	1.7	1.0–2.6
C	6.4	5.4–7.6	2.2	1.6–3.0	1.6	1.0–2.2	2.6	2.0–3.4
D	11.5	9.8–13.0	4.2	3.4–5.4	4.1	3.2–5.2	2.8	2.0–3.8
E	12.6	10.6–15.0	4.3	3.2–5.8	6.8	5.2–8.6	1.5	0.8–2.4

Note. Group A = controls; group B = pilots of domestic flights; group C = pilots of transequatorial flights; group D = pilots of transpolar flights; group E = retired pilots. B’ = monochromatid breaks; B’’ = isochromatid breaks; CI = 95% confidence interval.

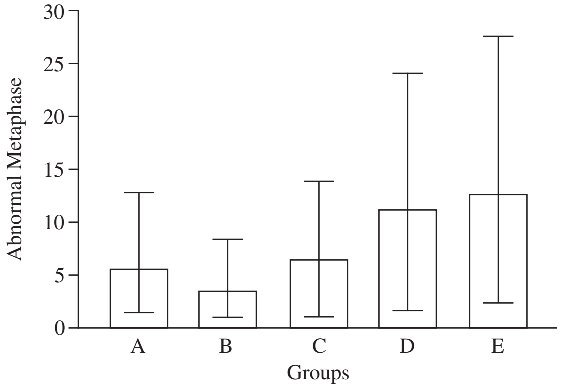

FIGURE 1. Frequency of abnormal metaphases in group A (controls), group B (domestic pilots), group C (transequatorial pilots), group D (transpolar pilots), and group E (retired pilots). Depicted data represent means and 95% confidence intervals.

were not the cause of the observed differences. No significant differences were observed for monochromatid breaks among all the studied groups (see Table 4 and Figure 2). However, monochromatid breaks with age tend to increase (even though this event occurred at no significant level) ($p < .06$; see Table 4 and Figure 2). The frequencies of isochromatid breaks in groups A, C, D, and E were significantly higher than those observed in group B ($p < .02$; see Table 4 and Figure 3). Analysis of dicentric chromosomes showed a significant increase when groups B, C, D, and E were compared with the control group (group A; $p < .01$). Groups C and D had a higher prevalence of dicentric chromosomes than groups B and E ($p < .05$). No differences were found between groups C and D or groups B and E (see Table 4 and Figure 4). Confounding factors (age and flight hours) did not explain the observed differences shown by the multivariate analysis.

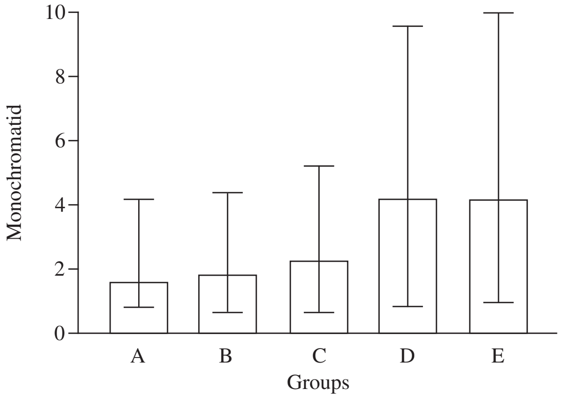

FIGURE 2. Frequency of monochromatid breaks in group A (controls), group B (domestic pilots), group C (transequatorial pilots), group D (transpolar pilots), and group E (retired pilots). Depicted data represent means and 95% confidence intervals.

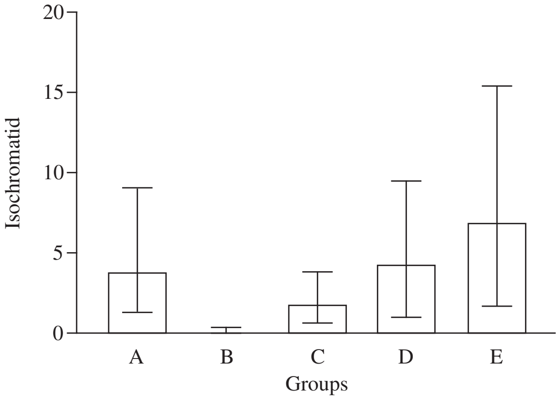

FIGURE 3. Frequency of isochromatid breaks in group A (controls), group B (domestic pilots), group C (transequatorial pilots), group D (transpolar pilots), and group E (retired pilots). Depicted data represent means and 95% confidence intervals.

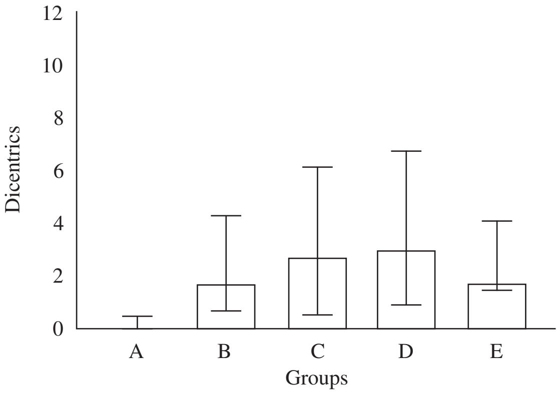

FIGURE 4. Frequency of dicentric chromosomes in group A (controls), group B (domestic pilots), group C (transequatorial pilots), group D (transpolar pilots), and group E (retired pilots). Depicted data represent means and 95% confidence intervals.

COMMENT

The amount of chromosomal damage found in blood samples of international Argentinean pilots was higher than that reported in previous articles.[23,26] However, the results obtained from previous studies showed some discrepancies. Whereas significant increases in chromosomal aberrations were found in air crew members of subsonic flights,[24–26] no differences were observed when the cytogenetic study targeted female cabin attendants.[20] On the other hand, high frequencies of chromosomal aberrations, mainly dicentric chromosomes, were found in Concorde pilots[21] and astronauts.[23]

Even though sample size was small, our study suggests that chronic exposure to low levels of cosmic radiation in aircraft members is related to the observed clastogenic effect. This exposure level could originate from at least 2 sources. First, most international flights from Argentina are transequatorial, traveling long distances at high altitudes. Second, about half of the pilots studied were routine crew members of the transpolar Buenos Aires–Auckland flight.

The number of dicentric chromosomes in human DNA is considered a biological dosimeter for radiation exposure.[21] The number of dicentric chromosomes found in active international crew members was higher than that in controls, pilots of domestic flight, and retired pilots, and ten-fold higher than that in laboratory controls.

An interesting finding is the persistence of exchange-type aberrations in retired aircrew members, even 10 years after exposure. Studies carried out 6 years after accidental exposure in Goianias, Brazil, pound a rapid decrease in the frequencies of dicentric and ring chromosomes was found in the highest exposed group (doses higher than 1 Gy). In individuals receiving less than 1 Gy, chromosomal aberrations remained unchanged over time. In the first case, the estimated average half-life of dicentric elimination was 110 days for the initial period after exposure (up to 470 days). In the second case, the half-life was 160 days for the period of 470 days.[5] The cytogenetic follow-up of air crews chronically exposed to low radiation doses, including cabin attendants, is an open field for further research. It has been demonstrated that the increase of exchange-type aberrations is an indicator of cancer risk which warrants further study.[29–31]

In conclusion, Argentine international pilots (especially transpolar pilots) showed a high incidence of chromosomal damage, with a concomitant increase in exchange-type chromosomal aberrations. These observations suggest that the chronic exposure to low levels of cosmic radiation in aircraft members would explain the observed clastogenic effect. Consequently, further studies are necessary to elucidate the mechanisms involved in the origin of chromosomal damage in air crews and its association with cosmic radiation exposure. Although the number of dicentric chromosomes in domestic pilots is lower than that in international pilots, they should be screened to evaluate their exposition risk. Results obtained in this study could contribute to the establishment of a new upper limit of cosmic radiation exposition to avoid or minimize the risk to air crew members. Finally, because we found a persistence of dicentric chromosomes in retired pilots, they should also be monitored for these type of aberrations over time.

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