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Normal Genetic Response to Gamma Irradiation in Familial Adenomatous Polyposis

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The present study, a co-operative project between three European institutes, was aimed at elucidating whether the *APC* gene in carriers of familial adenomatous polyposis coli (FAP) also causes some genetic sensitivity revealed by DNA damage and the yield of chromosome aberrations in peripheral blood lymphocytes exposed to gamma rays. In addition, it seemed of interest to study whether DNA repair is modified after irradiation of lymphocytes from FAP patients compared to controls. To this end, we have used the inhibition of the poly(ADP-ribose) polymerase (ADPRP) by 3-aminobenzamide (3ABA) and studied the effect of 3ABA on the frequency of DNA strand breaks and chromosome aberrations. The data indicate that FAP is not associated with an increased chromosomal sensitivity towards ionising radiation.

Key words: chromosomal aberrations, DNA strand breaks, gamma rays, poly(ADP-ribose) polymerase, familial adenomatous polyposis

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INTRODUCTION

MANY FACTORS influence the appearance of colon carcinoma [1] among which genetic predisposition such as familial adenomatosis polyposis coli (FAP) determined by the *APC* gene localised on chromosome 5 has received most attention. Several genetically transmissible defects leading to a predisposition to cancer have been shown to be associated with an increased sensitivity to mutagens. Mutagenic factors in food [2, 3] might, therefore, significantly contribute to the development of colon cancer in predisposed human carriers. Other mutagenic agents, such as ionising radiation, may play a similar role. Indeed, a significant increase in colon cancer has been found among the 93 000 survivors of the atomic bombs of Hiroshima and Nagasaki during the period 1958–1987 [4].

The present study, a co-operative project between three European institutes, aimed to elucidate whether the *APC* gene in carriers of FAP also causes some genetic instability revealed by DNA damage and the yield of chromosome aberrations in peripheral blood lymphocytes exposed to gamma rays. In addition, it seemed of interest to study whether DNA repair is modified after irradiation of lymphocytes from FAP patients compared to controls. To this end, we have used the inhibition of the poly(ADP-ribose) polymerase (ADPRP) by 3-aminobenzamide (3ABA) and studied the effect of 3ABA on the frequency of DNA strand breaks and chromosome aberrations. ADP ribosylation has been shown to be an important process in the DNA excision repair and is stimulated when DNA strand breaks are formed either directly, for example by ionising radiation, or via enzymatic reactions during DNA excision repair [5].

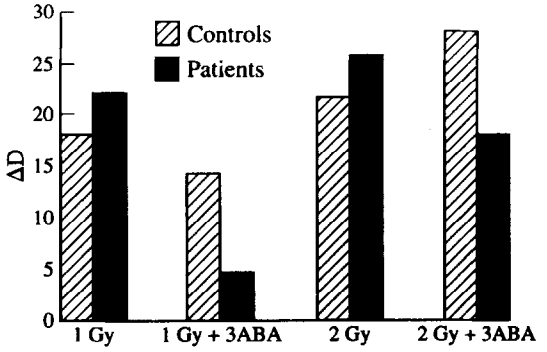

Figure 1. Induction of DNA breakage by ^{60}Co gamma radiation in controls and APC patients.

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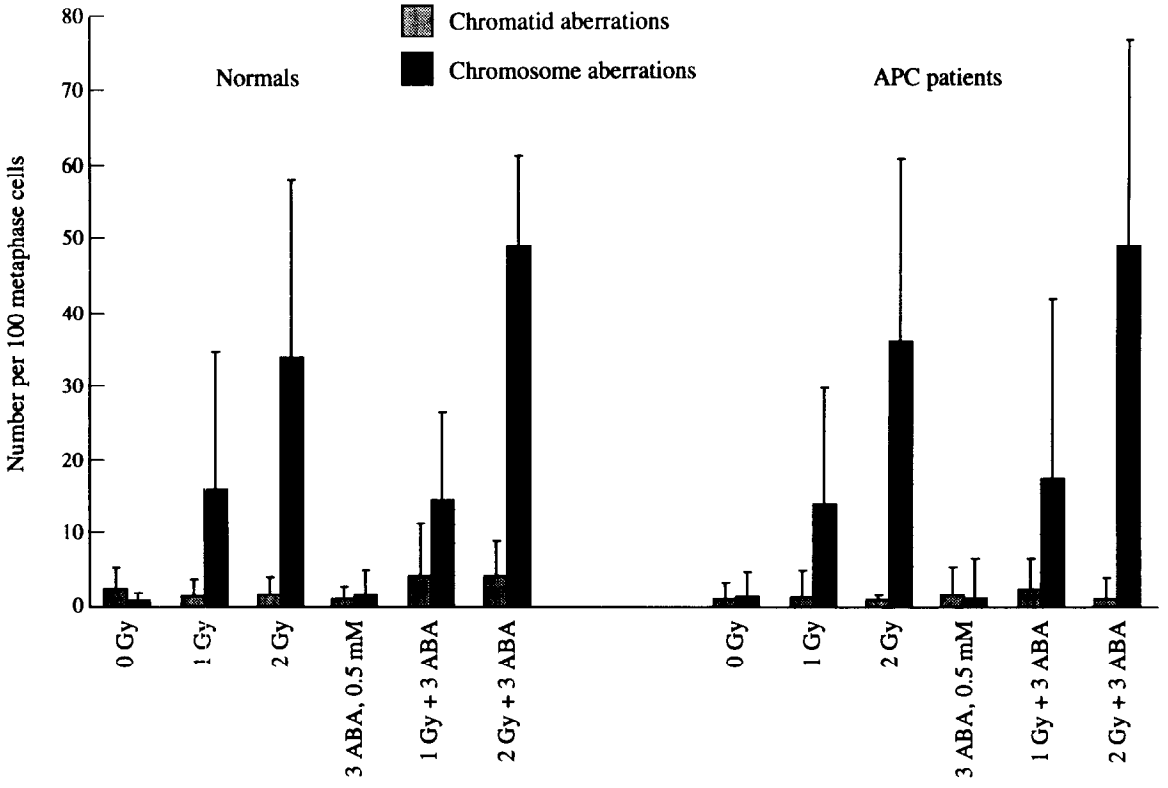

Figure 2. Structural aberrations in controls and APC patients in the absence and presence of 3ABA.

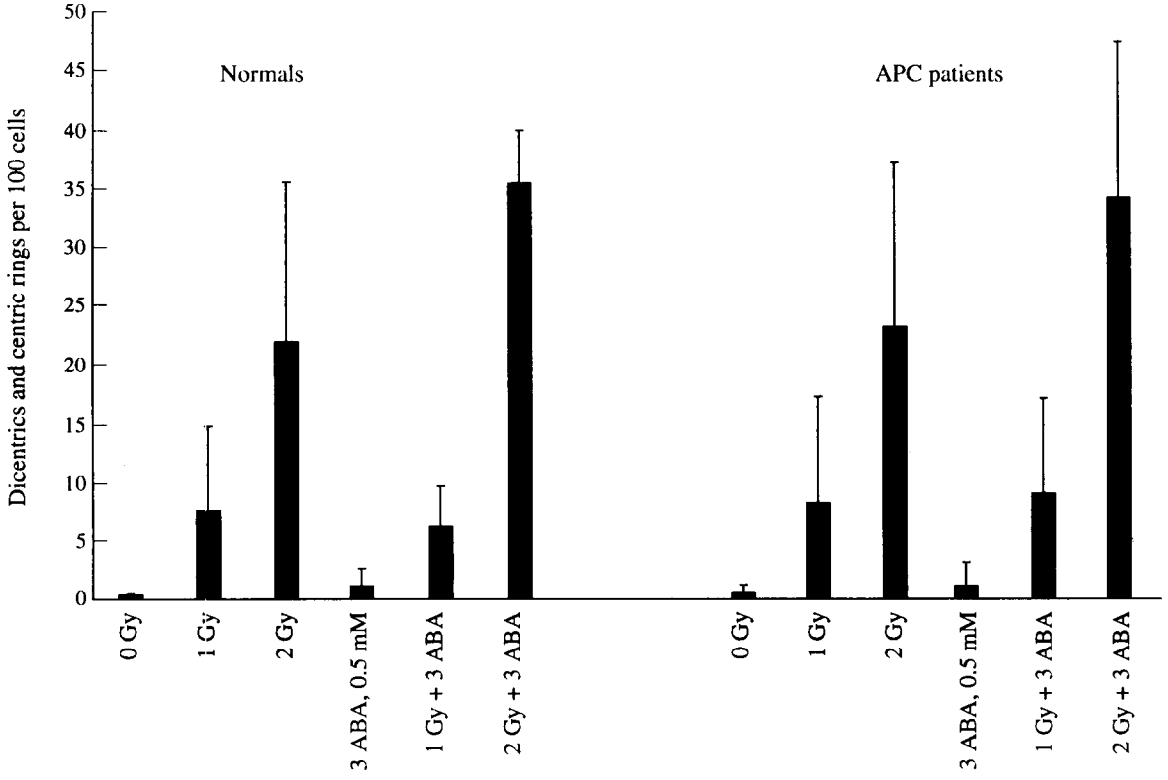

Figure 3. Distribution of dicentric and centric rings in controls and APC patients in the absence and presence of 3ABA.

Therefore, inhibition of this pathway by 3ABA has been used routinely to study inhibition of DNA repair after exposure to agents damaging DNA such as ionising radiation and alkylating agents [6]. It has, indeed, been found that patients predisposed to colorectal cancer, e.g. by FAP, have reduced DNA repair when assessed by ADPRP activity [7].

MATERIALS AND METHODS

In this co-operative study, blood samples from 11 FAP patients detected in Lisbon and 11 controls were exposed to 1 or 2 Gy of ^{60}Co gamma irradiation to determine chromosome aberrations. 8 FAP patients and 23 controls served to investigate DNA breakage after irradiation of the blood. The studies were

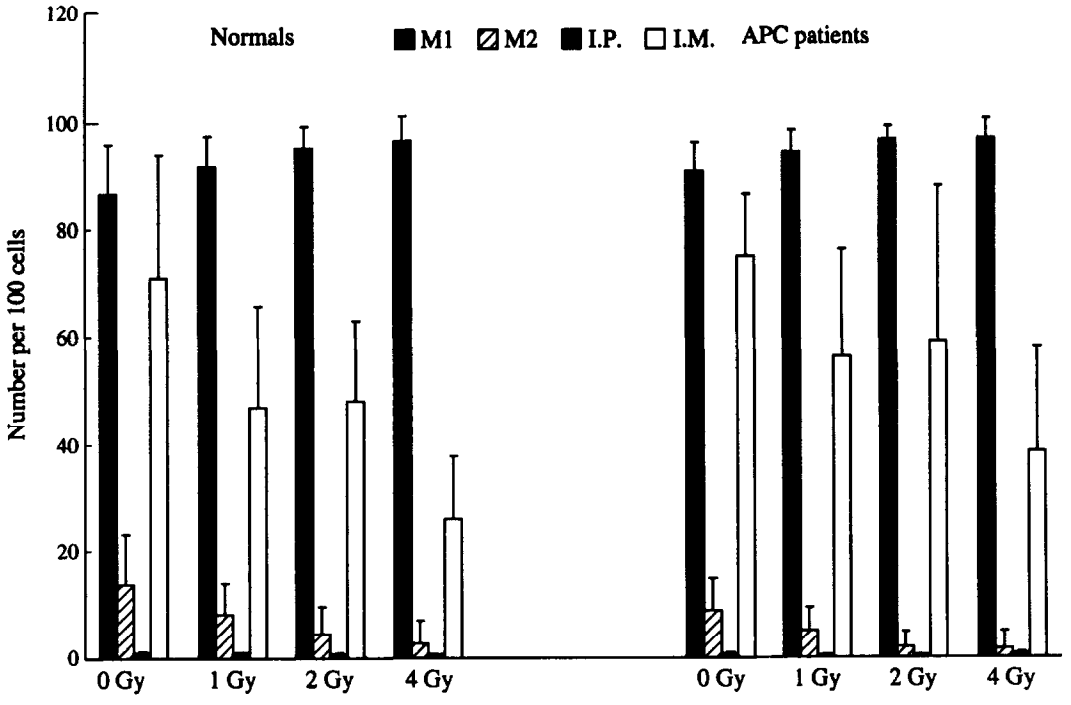

Figure 4. Cell kinetics and mitotic index in controls and APC patients.

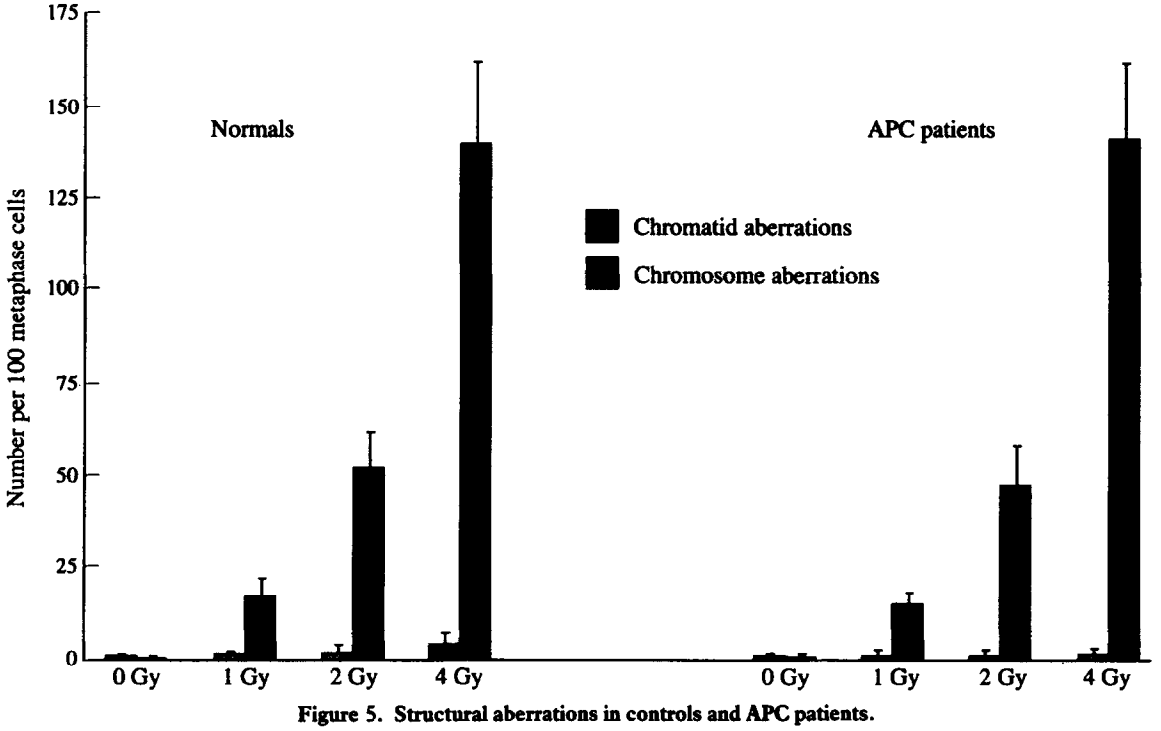

Figure 5. Structural aberrations in controls and APC patients.

carried out in the presence and absence of 3-aminobenzamide, an inhibitor of poly(ADP-ribose)polymerase. DNA breaks were determined on the basis of the fluorometric analysis of DNA unwinding (FADU method) as developed by Birnboim and Jevcak [8] and described in detail by Rueff and associates [9]. The results are expressed as ΔD, the difference between the percentage of double-stranded DNA (%D) in the untreated samples and %D in the treated samples (8). To determine chromosome aberrations, the blood samples were cultured in Ham's F-10 medium for 48 h and the metaphase slides were prepared by standard techniques. Two hundred cells were analysed from each individual and for each treatment. The 3ABA was dissolved in deionised water at a concentration of 0.1 M, sterilised through a Dynagard TM 0.2 μm syringe filter and added to the culture medium at a final concentration of 0.5 mM. The solutions were carefully protected from light. Structural chromosomal aberrations in irradiated lymphocytes from blood of 6 FAP patients, detected in Paris, and of 6 controls were also

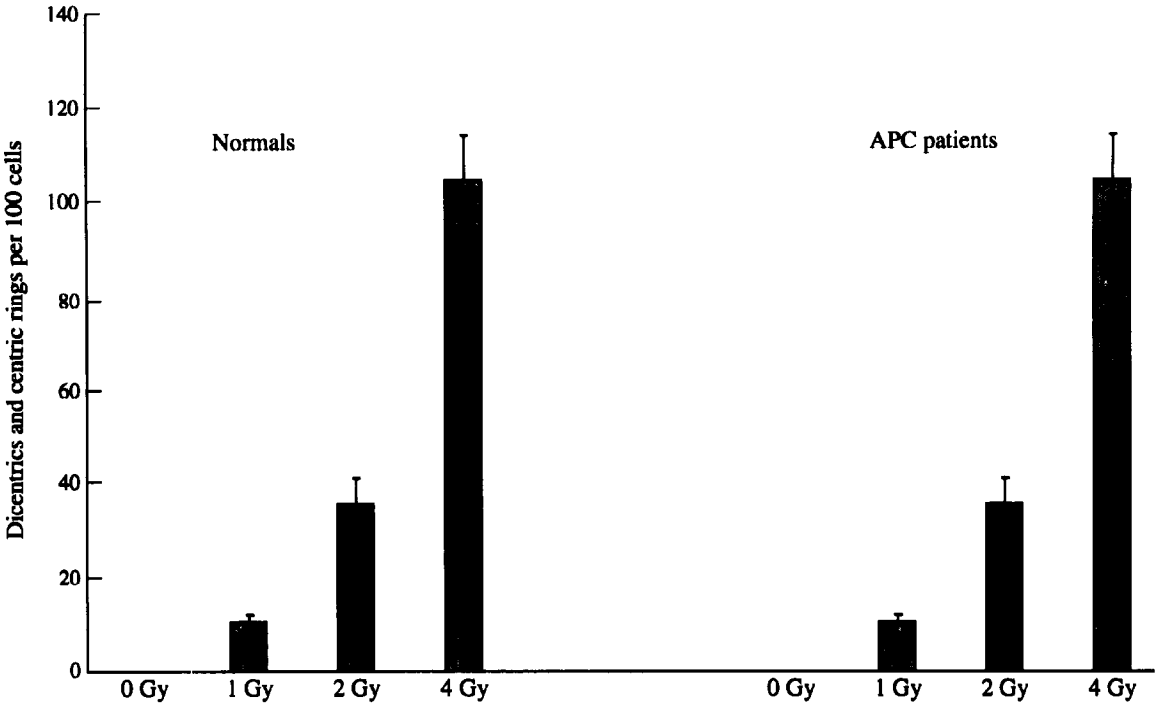

Figure 6. Distribution of dicentrics and centric rings in controls and APC patients.

studied in Brussels. The doses of ^{60}Co gamma rays were 1, 2 and 4 Gy. Cell kinetics of the lymphocytes (percentage of cells in M1, M2 and M3 phases) was investigated after 72 h culture using the bromo deoxyuridine technique [10].

RESULTS

The results summarised in Figures 1–3 (observations from Lisbon) and Figures 4–6 (observations from Brussels) do not reveal any difference in the reaction of lymphocytes to radiation and their DNA repair ability between blood from FAP patients and from controls. This is true for the yield and distribution of chromosome aberrations induced by different doses of ionising radiation as well as for the cell kinetics, mitotic index and induction of DNA breaks. Also, as expected [6], 3ABA increased the yield of chromosome aberrations after irradiation but this increase was about the same in FAP patients and controls. Poly(ADP-ribose) polymerase is a multifactorial enzyme which in the presence of DNA breaks attaches ADP-ribose from NAD to various nuclear proteins [11]. It has been suggested to participate in repair of γ-irradiated DNA, having a 'nick-protection' mechanism which may prevent the induction of chromosomal aberrations [12]. It is thus not surprising that our data showed a higher amount of chromosomal aberrations when the enzyme was inhibited by 3ABA. We have not addressed in the present work the possible instability in the progeny of irradiated cells many cells divisions after exposure, which cannot be ruled out. A limitation of this study to fully assess chromosomal sensitivity in FAP is the somewhat rather limited number of patients studied. Further data should be gathered to rule out the existence of chromosomal sensitivity in FAP.

DISCUSSION

The present results, thus, confirm to some extent several negative results reported in the literature. Ban and associates [13] studying the skin sensitivity of skin fibroblasts from patients with FAP, found no difference to controls with respect to DNA damage produced by X-rays or UV-radiation. More recently, Kakati and colleagues [14] also failed to observe significant differences in the averages of chromosome aberrations determined in cultured lymphocytes from FAP patients and controls without exposure or exposure to 300 rads ^{137}Cs gamma irradiation. Similarly, sensitivity to mitomycin-C was the same in FAP patients and controls [13, 15].

In conclusion, our data confirm that familial adenomatous polyposis coli is not associated with an increased chromosomal sensitivity towards mutagens such as ionising radiation.

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