Unscheduled DNA Synthesis in Mononuclear Leukocytes from Patients with Colorectal Polyps

Ronald W. Pero, Marianne Ritchie, Sidney J. Winawer, Melvin M. Markowitz, and Daniel G. Miller

ABSTRACT

The mononuclear leukocytes from peripheral blood samples of individuals with (n = 30) and without (n = 48) colonic polyps were examined for their abilities to carry out unscheduled DNA synthesis (UDS) induced by N-acetoxy-N-2-fluorenylacetamide (N-AcO-2-FAA). Individuals with polyps had significantly reduced UDS values compared to the nonpolyp group (P < 0.01). Furthermore, in a more comprehensive study, patients with hyperplastic polyps had N-AcO-2-FAA-induced UDS values not significantly different from control individuals who were asymptomatic and free from colonic disease as judged by complete colonoscopy. However, patients who had had adenomatous polyps in their large bowel had significantly reduced levels of N-AcO-2-FAA-induced UDS in their mononuclear leukocytes (P < 0.005). When N-AcO-2-FAA binding to DNA determinations were made in parallel and DNA repair proficiency indices were calculated (i.e., N-AcO-2-FAA-induced UDS/N-AcO-2-FAA binding to DNA), the patients with adenomatous polyps were still shown to be deficient in carrying out DNA repair synthesis. Since adenomatous polyps of the large bowel are considered the premalignant lesion for colorectal cancer, we postulate that reduced UDS may be a genetically sensitive marker that is useful in studying the mechanisms of genetic predisposition to colorectal cancer.

INTRODUCTION

A body of evidence now exists which supports the association of adenomas with cancer in the colon and rectum (1–5). Some of the work supporting this hypothesis is derived from studies of patients with FPC (2, 6, 7). These individuals possess an autosomal dominant gene which predisposes them to the production of numerous polyps and a high incidence of colorectal cancer. In addition, chromosome instability has been found in the lymphocytes, fibroblasts, and other cells of FPC patients (6, 8–12); whether there is also a distinct environmental determinant of FPC is still uncertain.

Recently, a marked reduction in DNA repair, measured as UDS, was demonstrated in both postoperative colon cancer patients and a well-defined population that was genetically predisposed to polyps and colorectal cancer; i.e., from FPC families (13). Since such a reduction in DNA repair appeared in genetically predisposed polyp-producing individuals, we wanted to investigate the possibility that it might not be specific only for cases with FPC but might also occur in patients who produce sporadic polyps. This study reports on the various degrees of DNA repair synthesis as they occurred among groups of patients who demonstrated various types of polyps and then compares them to an unaffected control population.

MATERIALS AND METHODS

Study I. Preliminary Investigation of Patients with and without Polyps. Patients with polyps (n = 30) were identified from among fecal occult blood-positive patients seen at the PMI-Strang Clinic and patients referred to the Gastroenterology Service at Memorial Sloan-Kettering Cancer Center. Controls (n = 48) were selected from individuals presenting themselves to the PMI-Strang Clinic for a comprehensive medical examination who were asymptomatic and had normal proctoscopies. All subjects were sampled during the period of October 1982 to May 1983, at the rate of 1 to 5 individuals per week. The sampled populations were all age-matched males, and they were not eliminated because of differences in smoking habits or blood pressure, since we considered this study as a preliminary investigation for evaluating the interindividual variation encountered in these populations, using N-AcO-2-FAA-induced UDS in mononuclear leukocytes as a probe. Radiolabeled N-AcO-2-FAA was not available to us during this part of the study, and so estimations of N-AcO-2-FAA binding to DNA were not carried out.

Study II. Patients with and without Histologically Defined Polyps. Only individuals who had a complete colonoscopy were entered into this part of the study. The colonoscopies were performed within 6 months of the sampling period, which ran from June 26 to August 28, 1984. Four groups of patients were discernible based on the diagnosis and pathological findings following colonoscopy. They were (a) individuals with colons free from any abnormal manifestations, designated “clean colons” (n = 5); (b) individuals with hyperplastic polyps (n = 7); (c) individuals with both hyperplastic and adenomatous polyps (n = 5); and (d) individuals with only adenomatous polyps (n = 6). Both males and females were accepted into this study, and all subjects were age-matched. In addition, the individuals under study were nontobacco, nonsmoking, and not taking any medications at the time of the study. Quantitative determinations of both N-AcO-2-FAA-induced UDS and N-AcO-2-FAA binding to DNA were carried out using mononuclear leukocyte samples from all of the aforementioned individuals who had had a complete colonoscopy.

Sampling Procedures. Heparinized vacutainers containing 143 U.S.P. of heparin per 10-ml tube were used to obtain 30- to 40-ml samples of peripheral venous blood. Controls were always sampled at the same time (i.e., within 2 days) as patients with benign bowel disease.

The heparinized blood was centrifuged at 100 × g for 10 min, and the platelet-rich plasma was removed with a sterile Pasteur pipet. Platelets were then removed from plasma by centrifugation at 400 × g for 25 to 30 min, and the resulting platelet-poor plasma was then used as a culture supplement during the estimation of N-AcO-2-FAA-induced UDS and N-AcO-2-FAA binding to DNA.
UDS in Patients with Polyps

$[^3H]N$-AcO-2-FAA binding to DNA. The blood cell pellet was diluted to its original volume with 0.9% NaCl solution (saline), and then the sample was layered onto a Ficoll-Isoaque cushion and spun at 400 $\times$ g for 25 to 30 min. The mononuclear leukocytes containing about 80% lymphocytes and 20% monocytes were isolated from the interphase zone of the gradient.

N-AcO-2-FAA-induced UDS. The quantitative assessment of UDS following a standardized in vitro exposure of human mononuclear leukocytes to N-AcO-2-FAA has been published elsewhere (14). In general, UDS was quantified biochemically after incubation of 5 $\times$ 10$^6$ cells with a 1.0 $\mu$M dose of N-AcO-2-FAA for 1 h at 37°C in 1% autologous plasma-supplemented Eagle’s minimum essential medium fortified with Hank’s salt solution. After exposure, the cells were incubated in fresh culture medium for an additional 17 h in the presence of 10 nM hydroxyurea and $[^3H]$dThd (10 $\mu$Ci/ml; Amersham Corporation, Arlington Heights, IL). The data were recorded as cpm $[^3H]$dThd per ug DNA minus the $[^3H]$dThd incorporated from hydroxyurea-suppressed background DNA synthesis.

The hydroxyurea-suppressed DNA synthesis values ranged from 82 to 334 cpm $[^3H]$dThd/ug DNA with a mean of 201 ± 11 (SE) for all of the sampled populations. There was also a small difference in Study II (P<0.05) between individuals with (173 ± 13) and without (225 ± 18) adenomatous polyps, but the N-AcO-2-FAA-induced UDS values were always 2- to 10-fold higher than the background level of hydroxyurea-suppressed DNA synthesis. Biochemical determinations were carried out in duplicate (i.e., usually involving 5 to 10 $\mu$g DNA/replicate) with reproducibility of about ±15% (14, 15).

N-AcO-2-FAA Binding to DNA. Binding studies were performed on parallel cultures identical to those used for UDS determinations. Approximately 5 $\times$ 10$^6$ mononuclear blood cells were suspended in 5 ml of Eagle’s minimum essential medium fortified with Hank’s salt solution and supplemented with 1% autologous plasma, and then the cells were exposed to 1 $\mu$M $[^3H]$N-AcO-2-FAA (950 mCi/mmol; Midwest Research Institute, Kansas City, MO) and incubated for 30 min at 37°C. The binding of N-AcO-2-FAA to DNA is maximum after 30 min, and so the level of DNA damage induction is equivalent to the conditions used for UDS determinations. After incubation, the cells were pelleted at 300 $\times$ g for 10 min and washed once with saline. The DNA was extracted and purified, and radioactivity was counted and quantified, as we have discussed previously (13, 14). The data were recorded as the average of duplicate biochemical determinations quantified as cpm $[^3H]$N-AcO-2-FAA incorporated per ug DNA. Reproducibility of N-AcO-2-FAA binding determinations is about ±10% (15).

DNA Repair Proficiency Index. The DNA repair proficiency indices for each of the sampled individuals were calculated by dividing the N-AcO-2-FAA-induced UDS value by the $[^3H]$N-AcO-2-FAA binding to DNA value.

RESULTS

The data relating effects on the estimation of N-AcO-2-FAA-induced UDS in mononuclear leukocytes to patients with polyps is presented in Table 1. It appears that individuals who have had polyps show significantly lower UDS values (P < 0.01) than controls who are asymptomatic and who have had negative proctoscopies. Since mononuclear leukocytes are non-target cells for colon cancer, then these data suggest that UDS in response to N-AcO-2-FAA may be a genetic marker for colon cancer. This interpretation is also consistent with the evidence that adenomatous polyps are considered to be the premalignant lesion for colon cancer (3).

The data in Study I were inconclusive because (a) estimates of N-AcO-2-FAA binding to DNA were not carried out, due to the lack of availability of radiolabeled N-AcO-2-FAA, and therefore, UDS values could not be corrected for differences in sensitivity to react with N-AcO-2-FAA by calculating DNA repair proficiency indices (13); (b) asymptomatic controls only had a proctoscopy and not a colonoscopy and, hence, at least some may have had polyps; (c) not all of the patients’ polyps had been histologically verified, preventing us from breaking down this group even further into hyperplastic and adenomatous polyp subgroups; and (d) because of the preliminary nature of this first study, individuals were not eliminated because of differences in blood pressure and smoking habits, both of which are known to affect UDS (14, 16, 17).

Therefore, a highly controlled prospective study was undertaken using mononuclear leukocytes as the indicator cells, in order to determine if a reduced UDS was associated specifically with patients having sporadic adenomatous polyps in their large bowel. Patients who had had a recent complete colonoscopy (i.e., within 6 months), were invited to the Digestive Disease Center at the PMI-Strang Clinic to give a blood sample for determination of both N-AcO-2-FAA-induced UDS and N-AcO-2-FAA binding to DNA. Since these patients had been recently colonoscopyed, we were then able to know precisely the condition of their colons. Only normotensive nonsmoking individuals who were not taking medications were accepted into the study.

We were able to distinguish four groups of patients with regard to the presence or absence of polyps. The data are presented for statistical analysis in Chart 1, where subjects with clean colons (n = 5) were combined with subjects having hyperplastic polyps (n = 7) and those having only adenomatous polyps (n = 6) were combined with those having both hyperplastic and adenomatous polyps (n = 5). Chart 1A clearly indicates that a reduced N-AcO-2-FAA-induced UDS segregated with patients who had had adenomatous polyps in their colons (P < 0.005). A reduced capacity for DNA repair synthesis was confirmed by the results of studying simultaneously N-AcO-2-FAA binding to DNA in 21 of the 23 patients under investigation. This has allowed us to calculate DNA repair proficiency indices (i.e., N-AcO-2-FAA UDS/N-AcO-2-FAA binding to DNA), which estimates the levels of DNA repair synthesis (i.e., UDS) per unit of DNA damage induced by N-AcO-2-FAA. Chart 1B verifies that the reduced UDS seen in Chart 1A reflected a true deficiency in DNA repair synthesis for those patients who had had adenomatous polyps in their colons, since they also had significantly reduced DNA repair proficiency indices (P < 0.01).

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>N-AcO-2-FAA-induced UDS (cpm $[^3H]$ dThd/ug DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (no polyps)</td>
<td>n = 48</td>
<td>M</td>
<td>55.3 ± 1.3$^a$</td>
<td>441 ± 24</td>
</tr>
<tr>
<td>Cases with polyps</td>
<td>n = 30</td>
<td>M</td>
<td>57.5 ± 2.0$^b$</td>
<td>351 ± 18</td>
</tr>
<tr>
<td>t-test</td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

$^a$ Mean ± SE.

$^b$ NS, not significant.
UDS IN PATIENTS WITH POLYPS

Chart 1. Levels of N-AcO-2-FAA-induced UDS and the DNA repair proficiency indices calculated in the mononuclear leukocytes from individuals having various histological types of polyps in their colons. All individuals participating in this study had a complete colonoscopy within 6 months of the time of sampling. The polyps were removed during colonoscopy, and the individuals were divided into groups based on the histology of the polyps. Peripheral blood samples were collected and processed, and the levels of N-AcO-2-FAA-induced UDS and N-AcO-2-FAA binding to DNA were determined in parallel as described in “Materials and Methods.” ○, clean colon controls (n = 5; mean age = 49.8 ± 5.1 years; mean UDS = 950 ± 147); ●, hyperplastic polyps (n = 7; mean age = 56.3 ± 4.8 years; mean UDS = 1064 ± 191); ▼, adenomatous polyps (n = 6; mean age = 59.0 ± 5.7 years; mean UDS = 596 ± 99); ▼, adenomatous and hyperplastic polyps (n = 5; mean age = 61.8 ± 1.3 years; mean UDS = 419 ± 85). The control groups (clean colons and hyperplastic polyps) were compared to the adenoma groups (adenomas with and without hyperplastic polyps) by a Student’s t-test. N-AcO-2-FAA binding to DNA values were not determined for 2 individuals because of lack of cells and, therefore, DNA repair proficiency indices could not be calculated. Means are given; bars, SE.

DISCUSSION

Measurements of DNA repair synthesis (UDS) induced by chemical agents such as N-AcO-2-FAA reflect not only the ability of the cell to synthesize new DNA to repair DNA lesions but also any metabolic differences within the cell that lead to an altered potential for DNA damage induction (i.e., activation, transport, or uptake of N-AcO-2-FAA). N-AcO-2-FAA-induced UDS is thus potentially regulated in 2 steps: (a) the first involves the introduction of N-AcO-2-FAA damage into cellular DNA, and it may be quantified by measuring the covalent binding of radiolabeled N-AcO-2-FAA to cellular DNA; and (b) the second is the repair of the DNA lesions introduced in the first step which can be quantified as UDS by the incorporation of [3H]dThd into cellular DNA. Since N-AcO-2-FAA UDS is directly dependent of N-AcO-2-FAA covalent binding to DNA (13), then the index of N-AcO-2-FAA UDS/N-AcO-2-FAA binding to DNA is a convenient estimate of DNA repair proficiency. Using this estimate, we have determined that UDS may be a genetically sensitive marker for colorectal cancer and therefore may play a role in predisposing individuals to this type of cancer.

Evidence is accumulating that individuals genetically predisposed to colorectal cancer have an increased sensitivity to acquire DNA damage. So far, increases in tetraploidy (2), in sister chromatid exchanges (9, 11), in chromosome aberrations (6, 10, 12), and in levels of covalent binding to 4-nitroquinoline-1-oxide (18) have been demonstrated in fibroblasts from patients with FPC or Gardner’s syndrome. Moreover, our earlier study (13), together with the data reported here (Table 1 and Chart 1), have shown that individuals with colorectal cancer, FPC, or even just a sporadic adenomatous polyp have a reduced capacity for DNA repair synthesis. Therefore, it seems quite possible that individuals manifesting an adenomatous polyp, the recognized premalignant lesion for colorectal cancer, have a basic genetic predisposition which renders them more susceptible to environmental mutagens/carcinogens because they cannot repair DNA lesions as efficiently as unaffected individuals can. There is scientific precedence for such reasoning, since cancer-prone conditions such as xeroderma pigmentosum, Bloom’s syndrome, ataxia telangiectasia, and Fanconi’s anemia are known to accumulate cytogenetic abnormalities, and the mechanisms involved are suspected to be defective DNA repair (19).

The presence of an adenomatous polyp constitutes a biological marker in the general population for colorectal cancer with or without a family history (3). Familial polyposis and Gardner’s syndrome are good examples of the importance of adenomatous polyps as risk indicators in the genetic predisposition of patients to colorectal cancer. However, this study extends our earlier findings of DNA repair deficiency in genetically predisposed patients with FPC to also include patients with sporadic adenomatous polyps which may not necessarily express a known mode of inheritance. This point may have special relevance when one is trying to establish a general marker for colorectal cancer.
this regard, it would be important to determine ODC activity in patients with adenomatous polyps, since a recent report (20) showed elevated ODC in colonic mucosa of at-risk members of families with familial polyposis. Whether ODC and UDS measurements have common components or are otherwise biologically related remains to be determined. However, it is interesting to observe that ODC is activated by oxygen radicals that can damage DNA (21), so, if DNA damage accumulated because of deficiency in DNA repair, ODC would be increased.

ACKNOWLEDGMENTS

We thank Julie Powell and Margaretha Lund-Pero for their expert technical assistance.

REFERENCES

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