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Increased constitutional chromosome sensitivity to bleomycin in patients with hereditary non-polyposis colorectal cancer (HNPCC)

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Abstract. It has been suggested that mutagen sensitivity is a constitutional factor which may be useful in identification of patients with an increased risk for the development of tumors. In this study, the chromosome sensitivity to bleomycin was measured according to HSU in patients with hereditary non-polyposis colorectal cancer (HNPCC), sporadic colorectal cancer and in control persons with no tumor history in family. In vitro lymphocytes were exposed to bleomycin according to HSU and chromosomal damage was quantified by scoring breaks of 100 cells. A significant difference ($P < 0.01$) in the mean number of breaks per cell (b/c) was found between HNPCC patients (0.59 ± 0.14; $n = 12$; mean age 55.4 yrs) and control individuals (0.35 ± 0.13: $n = 12$; mean age 55.8 yrs). In contrast, patients with sporadic colorectal cancer showed a mean b/c value of 0.43 ± 0.14 ($n = 14$; mean age 63.4 yrs) which was not significantly higher than that in control individuals for this group (0.42 ± 0.15; $n = 14$; mean age 63.1 yrs). Selenium protected lymphocytes of HNPCC patients against bleomycin activity in vitro.

Key words: bleomycin, chromosome sensitivity, HNPCC.

Introduction

Host susceptibility to the genotoxic effects of environmental mutagens varies. Cytogenetic experiments have shown quantitative variations in susceptibility to mutagen-induced genetic damage (HSU et al. 1989). It is becoming increasingly accepted that for the occurrence of more common cancers an intrinsic latent or hidden susceptibility, which becomes apparent only after

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mutagenic exposure, is an important underlying factor (WIENCKE, SPITZ 1994). There are several cytogenetic assays of in vitro mutagen sensitivity (WIENCKE, SPITZ 1994). HSU has developed a relatively simple assay in which the frequency of chromosomal breakage is induced by in vitro exposure to the radiomimetic drug bleomycin (HSU 1987, HSU et al. 1985). In studies of patients with medullar thyroid, head/neck, lung and colon cancers bleomycin sensitivity was reported to be a significant risk factor for these cancers. (CHERRY, HSU 1983, HSU et al.1989). It seems that mutagen sensitivity assay according to HSU may be useful in identification of familial susceptibility to cancer because in bleomycin sensitive patients with tumors the features characteristic of hereditary cancers such as early onset, second primaries and familial aggregation of tumors, are occurring more frequently (HSU et al. 1989, SCHANTZ et al. 1990, BONDY et al. 1993).

These preliminary observations performed mainly in studies of head and neck or lung cancers have to be confirmed by further investigations. Herein we present results of bleomycin sensitivity assay in patients with HNPCC and sporadic colorectal cancer.

Material and methods

Subject

Twelve patients (7 females and 5 males) with colorectal cancer (CRC) fulfilling ICG-HNPCC criteria (VASEN et al. 1991, KŁADNY et al. 1995) were included into the HNPCC group. The average age in this group was 55.4 years (range from 29 to 73 yrs). One person from this group was smoking cigarettes (~20 cigarettes per day for 10 years).

Fourteen patients (8 females and 6 males) had CRC which was the only malignant tumor reported in the family and these persons were included into sporadic CRC group. The average age in this group was 63.4 (range from 39 to 74 years). Five persons from this group were smoking cigarettes – ~20 cigarettes per day for more than 20 years. Blood samples were obtained in a period of 1 month to 5 years after surgical resection of tumors. Patients who underwent chemo- or radiotherapy were not included into the studied group. Control persons consisted of age, sex and habits matched healthy volunteers and hospitalised persons without tumors and with no tumor history in their families. The average age of control persons for HNPCC group was 55.8 years (range from 30 to 81 years). The average age of control persons for sporadic CRC group was 63.1 years (range from 43 to 75).

Bleomycin sensitivity assay according to Hsu

Human peripheral blood lymphocytes were cultured at 37°C for 72 hr. One half ml of heparinized whole blood in 4.5 ml RPMI 1640 medium with L-glutamine phytohemagglutinin (LF-7, Biomed, Kraków) and 15% foetal calf serum. Culture of whole blood was started the same day it was obtained. Duplicate cultures of each subject were used. 0.03 U/ml bleomycin (Nippon Kayaku) was added 4-5 hours before cell harvest. Cells were arrested in metaphase by adding colcemid (Gibco) (0.2 μg/ml of culture) 1 hour before harvesting. Then, conventional air-dried preparations were made. All slides were coded and stained with Giemsa without banding. Reading was done with 50 metaphases per culture. All chromatid aberrations recorded were frank chromatid breaks or exchanges. Chromatid gaps, attenuated regions or metaphases with extensive breaks were disregarded. Each chromatid break was recorded as one break point and each chromosome break exchange figure was recorded as two break points. The frequency of breakage was expressed as breaks per cell (b/c).

Analysis of selenium influence on bleomycin effects

In these analyses 0.1 μg/ml of sodium selenite (Fluka) was added to cell culture medium at the beginning of the experiments.

Statistical analyses

To compare the number of b/c between the groups the Students t-test was used.

Results

The mean b/c values, standard deviations (SD) and percentage of patients with a b/c > 0.5 in the studied and control groups are summarised in Table 1.

A significant difference in the mean number of b/c was found between HNPCC patients (0.59 ± 0.14) and control individuals (0.35 ± 0.13; $P < 0.01$). There were 8 of 12 (66.7%) HNPCC patients and only 1 of 12 (8.3%) of control individuals with b/c > 0.5.

In contrast, patients with sporadic CRC showed the mean b/c value of 0.43 ± 0.14 which was not significantly higher than that of the control individuals for this group (0.42 ± 0.15; $P > 0.67$).

Selenium protected lymphocytes from HNPCC patients against bleomycin activity in vitro. After supplementing tissue culture media with 0.1 μg/ml

Table 1. Bleomycin-induced chromosomal damage in the different subject groups

Subject group	n	Breaks per cell $\bar{x} \pm SD$	Percentage of patients with b/c < 0.5
HNPCC	12	0.59 ± 0.14*	66.7
Controls for HNPCC	12	0.35 ± 0.13	8.3
Sporadic CRC	14	0.43 ± 0.14**	28.6
Controls for sporadic CRC	14	0.42 ± 0.15	28.6

* significantly different from controls; $P < 0.01$
** nonsignificantly different from controls; $P > 0.67$

of Sodium Selenite the mean b/c value (0.39 ± 0.14) of patients with b/c > 0.5 (2 of 12; 16.6%) was similar to that of the control individuals for the HNPCC group (0.35 ± 0.13; 1 of 12 cases – 8.3% with b/c > 0.5) (Table 2).

Table 2. The influence of selenium on bleomycin-induced chromosomal damage (mean ± SD) in the group of patients with HNPCC

Subject group	n	Breaks per cell $\bar{x} \pm SD$	Percentage of patients with b/c < 0.5
HNPCC	12	0.59 ± 0.14*	66.7
HNPCC + Selenium	12	0.39 ± 0.14	16.6
Controls for HNPCC	12	0.35 ± 0.13	8.3

* significantly different from controls; $P < 0.01$

Discussion

The HNPCC susceptibility genes have been identified recently on the chromosomes 2p – MSH2 gene (FISHEL et al. 1993), 3p – MLH1 gene (PAPADOPOULOS et al. 1994), 2q – PMS1 gene and 7p – PMS2 gene (NICOLAIDES et al. 1994). Mutations of these genes are major, but not the only factor determining development of tumors in families with HNPCC.

Results of our studies suggest that the kind of genomic instability which becomes apparent after exposure of peripheral blood lymphocytes to bleomycin is another inherited feature involved in carcinogenesis of HNPCC. We found

that constitutional chromosome sensitivity to bleomycin in patients with HNPCC is increased but is not significantly altered in patients with sporadic CRC. Increased sensitivity to bleomycin in patients with CRC was reported earlier by HSU et al. (1989). These autors did not diagnose hereditary syndromes such as HNPCC in their patients, which makes it impossible to compare their results with presented in this paper. Though this study was carried out on a small material the strength of the association between HNPCC and chromosome sensitivity is impressive and suggests a promising avenue for future research.

HNPCC genes are mismatch repair genes which are capable of recognising abnormal base pairs and correcting the sequence on one DNA strand to retrieve a normal A-T or C-G pairing. In colorectal tumors in carriers of mismatch repair gene mutations there is an increased damage of dinucleotide repeats which is molecularly manifested as the so-called microsatellite instability (RHYU 1996).

Genotoxic effects of bleomycin result from the formation of single-strand breaks, double-strand breaks and apurinic/apyrimidimic sites containing oxidised deoxyribose moieties (STUBBE, KOZARICH 1987). This DNA damage may be induced by hydroxyl radicals generated via free-radical-mediated reactions or possibly directly by activated bleomycin (AN, HSIE 1993). The chromosomal breakage, which is visible as an endpoint in the bleomycin sensitivity assay, represents only a fraction of the total mutation output. Most gene mutations are not microscopically detectable. Bleomycin sensitivity may consist of multiple underlying factors at several levels such as damage recognition, cell cycle arrest, DNA-repair enzymes and others (PANDIDA, HITTELMAN 1995). It cannot be excluded that mutations in mismatch repair genes are responsible for increased bleomycin sensitivity observed in our patients with HNPCC. Hypersensitivity to bleomycin has been found in patients with such tumors as medullary thyroid, lung and head/neck carcinomas where constitutional mutations in mismatch repair genes were not noted. Thus it is possible that patients with HNPCC carry a deficiency of additional DNA maintenance mechanism, different from impaired mismatch repair genes function.

Constitutional deficiency of DNA maintenance mechanism in patients with HNPCC was also suggested by PERO et al. (1983) who analysed the level of unscheduled DNA synthesis after exposure to N-acetoxy-N-2-fluorenylacetamide and by PARSHAD et al. (1983) who reported increased radiosensitivity in a spectrum of familial cancer syndromes including one family with multiple colon cancers. However BENDER et al. (1988) found that patients with familial non-polyposis colon cancer do not have increased sensitivity to irradiation and

such mutagens as N-methyl-N^1-nitro-N-nitrosoguanidine and mitomycin C. Current evidence indicates that generalised – it is to any mutagen – chromosomal sensitivity is not a common feature of cancer susceptibility. A high degree of mutagen specificity indicates rather that discrete sensitivities to specific classes of mutagens may play an important role in tumori genesis. Therefore, patients with HNPCC may be sensitive only to some particular mutagens. Bleomycin is a good candidate to be one of them. Susceptibility to chromosomal mutations is probably just one of probably numerous mechanisms involved in human risk for HNPCC. Furthermore, expression of chromosomal damage in peripheral blood lymphocytes is not likely to represent relevant alterations in cells undergoing changes in some cases. Nevertheless, we can see that in vitro exposure of lymphocytes to bleomycin and subsequent enumeration of cytogenetic alterations provide a promising biomarker to investigate HNPCC risk.

The process of carcinogenesis may be delayed or inhibited through the administration of synthetic or natural compounds such as, for instance, anti-oxidants or vitamins (MOON et al. 1983, De FLORA et al. 1991, KUNE et al. 1993). It cannot be excluded that environmental factors which reduce the level of bleomycin-induced chromosomal instability will be efficient protective agents for tumor development in families with HNPCC. In our studies we found that in vitro sodium selenite in doses recommended as non-toxic, protected lymphocytes of patients with HNPCC against bleomycin-induced chromosomal damage. Selenium supplementation practices has become very popular in these last decade (NEVE 1993). Selenium is recognised as an interesting pharmacological agent with potential anticarcinogenic properties (SHAMBERGER, RUDOLPH 1966, SHAMBERGER 1970, ZAJĄCZEK et al. 1987, Schrauzer 1992). Our studies indicate that one of possible pathways for protective activity of selenium is through inhibition of mutagen-induced DNA damage.

The extension of given in this work analyses on selenium influence on chromosomal instability seems to be justified. If additional studies confirm these preliminary results on a larger material and selenium protective effects are found also in vivo in animals with increased bleomycin sensitivity, a large chemoprevention trial in HNPCC families can be conducted.

Conclusion

Our preliminary data suggest that bleomycin test may be useful in management of families with HNPCC by detecting high risk individuals and by

monitoring anticarcinogenic effects of some agents described to be useful in chemoprevention.

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