Study on carcinogenesis in chronic cholecystitis

Zimnoch L[1], Szynaka B[1], Kupisz A[2]

[1]Department Medical Pathomorphology [2]District General Hospital, Medical University of Białystok

Abstract

Not only bile but also chronic cholecystitis may play a role in gallbladder carcinogenesis. Numerous studies have revealed a close correlation between chronic inflammation and neoplasia. The experiments were conducted on paraffin sections, obtained from 377 surgically resected gallbladders with chronic cholecystitis. Immunohistochemical reaction was conducted on deparaffinized sections, using a monoclonal antibody against 8-hydroxydeoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage. An increase was found in the expression of 8-hydroxydeoxyguanosine in chronic cholecystitis. The level of 8-OhdG expression is associated with inflammation intensity and disease duration. DNA damage, observed in chronic cholecystitis. indicates a correlation between chronic inflammation and gallbladder carcinogenesis.

Key words: chronic cholecystitis, carcinogenesis, oxidative DNA damage, 8-hydroxydoxy-guanosine.

Introduction

Gallbladder carcinogenesis is related to bile mutagenic activity, although a number of studies ascribe an important role to chronic cholecystitis. There are well known examples of a close association between chronic inflammation and carcinogenesis in some organs, including ulcerative colitis and carcinoma of the colon, Helicobacter pylori-related gastritis and gastric cancer, viral hepatitis and hepatic cancer [1, 2, 3].

The likely mechanism of inflammation effects on carcinogenesis is associated with oxidative DNA damage [4]. Cytokines, released by inflammatory cells, destroy DNA and can inhibit its repair [5].

ADDRESS FOR CORRESPONDENCE:
Lech Zimnoch
Department Medical Pathomorphology
Medical Universtiy of Białystok
Waszyngtona 13, 15-269 Białystok, Poland
e-mail: lech@amb.edu.pl

Material and methods

The study was conducted on paraffin sections, obtained from 377 surgically resected gallbladders with chronic cholecystitis, at the District General Hospital in Białystok, within a five-year period (1999-2003). The control group consisted of normal gallbladder specimens, collected from carcinoma patients.

The analysed parameters included: the gender, the age (age groups: 1/0 - 30 years, 2/31-50 years, 3/ over 51 years), disease duration (1/ up to 2 years, 2/ 2-5 years, 3/ over 5 years), the size and number of gallstones (1/ no gallstones, 2/ single, large, 3/ varying in size, 4/ small, a large number), and the degree of inflammation, according to the following scale: I^0 - several lymphocytes (<20) in 10 large fields of vision in the lamina propria of the mucosa, II^0 - intensive inflammatory infiltration - 20-100 lymphocytes in 10 large fields of vision, III^0 - hyper-intensive inflammatory infiltration >100 lymphocytes in 10 large fields of vision without lymph nodules, IV^0 - hyper-intensive inflammatory infiltration with 2-3 lymph nodules in 10 large fields of vision.

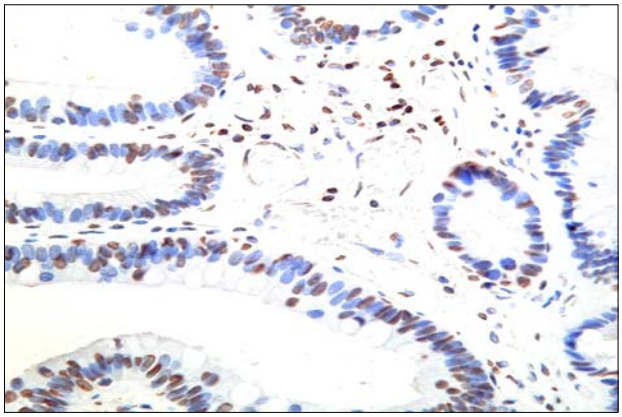
The deparaffinized sections, obtained from the archival specimens, stored at the Department of Pathomorphology, were immunohistochemically investigated, using a monoclonal antibody against 8-hydroxydeoxyguanosine (8-OhdG), (Oxis) (dilution 1:200) with LSAB kit (Dako) and methods recommended by Seki et al. [6].

The expression of 8-OhdG (8-OhdG index) was determined by counting the number of positive-cells among 200 epithelial cells of gallbladder mucosa (the mean of two calculations at 200x magnification for each case).

Table 1. 8-0HdG index in relation to the gender, the age, disease duration, the number of gallstones and inflammation degree.

Statistical multiple regression	Dependent variable regression: 8-0HdG INDEX R= .30900511 R2= .09548416 Corrected. R^2= .08081633 F (6.370)=6.5098 p< .00000 Standard estimation error: 7.5326					
N=377	BETA	St. Error BETA	**B**	St. Error B	t (370)	Level p
Gender	.081484	.049724	1.736097	1.059412	1.638736	.102118
Age	.010475	.051901	.133829	.663088	.201827	.840163
Disease duration	.206034	.053915	1.769343	.463002	3.821460	.000156
No of gallstones	.008838	.063727	.080779	.582468	.138684	.889776
Inflammation intensity	.216200	.049948	2.192183	.506457	4.328467	.000019

Figure 1. Expresion 8-hydroxydeoxyguanosine in chronic cholecystitis. Immunohistochemical reaction. Mag. x 400.



Multiple linear regression was used for statistical analysis, and p<0.005 was considered to be the level of significance.

Results The research material was obtained from 316 women and 61 men. The age group 1 (0-30 years) included 24 patients, the age group 2 (31 - 50 years) 13, and the age group 3 (over 51 years) - 217 patients. The disease duration: up to 2 years referred to 208 patients, from 2 to 5 years - 44 patients and over 5 years - 125 patients. No gallstones were found in 13 patients, 53 patients had single large gallstones, in 53 - gallstones varied in size and, in 192 - they were small and numerous. I^0 inflammation was noted in 90 patients, II^0 in 162, III^0 in 120 and IV^0 in 5 patients.

The performed immunohistochemical investigations revealed a varied expression of 8-hydroxydeoxyguanosine (8-OhdG) in the epithelial cells of gallbladder mucosa, ranging from very few coverage epithelial cells to a large number of cells. In some fields of vision, most cell nuclei of the surface epithelium and glandular tubes, lying underneath, showed a strong nuclear expression. In such cases, also lymphoidal cells in inflammatory infiltrations were positive for 8-OhdG (ryc.1). In the control group, the reaction was negative.

Statistical analysis showed a statistically significant difference between the level of 8-0HdG expression in gallbladder mucosal epithelium and the disease duration and inflammation degree (p< 0.0005). The remaining correlations, concerning the 8OhdG index and the gender and age, as well as the number and size of gallstones, showed no statistical significance (Table 1.).

Discussion

The immunohistochemical investigation, which employs antibody against 8-OhdG, serves to visualize oxidative DNA damage in human tissues and is considered a biomarker of considerable specificity [4]. Similarly as Seiko et al. [6], we found a correlation between the increase in the 8-0HdG index in gallbladder mucosal epithelium and inflammation degree. We also noted that the reaction intensity increased statistically significantly with the disease duration. The fields, where 80HdG expression in epithelial cells was markedly enhanced, also exhibited substantial lymphocytic infiltration. As similar observations have been reported by other authors, it can be assumed that inflammatory infiltration is the source of oxidative stress for gallbladder mucosal epithelial cells [7]. According to many authors, oxidative DNA damage plays a significant role in carcinogenesis [8]. Free oxygen radicals can be the major factors in promoting gene sequence instability, while inflammatory cytokines cause DNA damage and may inhibit the general mechanism of DNA repair. Although the precise role of 8-0HdG is unclear, in vitro studies have revealed that 8-0HdG accumulation in cellular DNA is mutagenic and carcinogenic; it is of great predictive significance for carcinogenesis in the breast [9].

Conclusions

In chronic cholecystitis, the expression of 8-hydroxydeoxyguanosine, a biomarker of oxidative DNA damage, is increased. The expression of 8-OHdG is associated with inflammation degree and disease duration. DNA damage, observed in chronic cholecystitis, indicates a correlation between cholecystitis and cancerogenesis.

References

1. Asaka M, Dragosics BA. Helicobacter pylori and gastric malignancies. Helicobacter, 2004; 9 Suppl 1: 35-41.

2. Seidelin JB. Colonic epithelial cell turnover: possible implications for ulcerative colitis and cancer initiation. Scand J Gastroenterol, 2004; 39: 201-11.

3. Poduri CD. Hepatitis C virus (HCV)--a review molecular biology of the virus, immunodiagnostics, genomic heterogeneity and the role of virus in hepatocellular carcinoma. Indian J Exp Biol, 2003; 4: 549-62.

4. Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroksydeoksyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutat Res, 1977; 387: 147-163.

5. Beckman KB, Ames BN. Oxidative decay of DNA. J Biol Chem, 1997; 272: 19633- 36.

6. Seki S, Kitada T, Yamada T, Sakaguchi H, Nakatani K, Onoda N, Satake K. Immunohistochemical detection of 8-hydroksydeokdyguanosine, a marker of oxidative damage, in human chronic cholecystitis. Histopathology, 2002; 40: 531- 8.

7. Feig DI, Reid TM, Loeb LA. Reactive oxygen species in tumorigenesis. Cancer Res, 1944; 54: 1890s - 94s.

8. Floyd RA. The role of 8- hydroksydoksyguanosine in carcinogenesis. Carcinogenesis, 1986; 7: 1849-51.

9. Musarrat J, Aresina-Wilson J, Wani AA. Prognostic and aetiological relevance of 8-hydroksydoksyguanosine in human brest carcinogenesis. Eur J Cancer, 1996; 32: 1209-14.