Measurement of Gut Diamine Oxidase Activity

Diamine Oxidase as a New Biologic Marker of Colorectal Proliferation?

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The close connection between polyamine metabolism and colonic epithelial cell proliferation has been reported in numerous studies.[1–3] Especially ornithine decarboxylase (ODC), the enzyme catalyzing the first step in the polyamine biosynthesis and producing putrescine (P), has already been intensively examined.[3–5] As the precursor of spermidine and spermine, P regulates the synthesis of these polyamines by its actual concentration. P is degraded by diamine oxidase (DAO).[6–8] Thus, DAO determines local P concentrations, and the extent of spermidine and spermine generation is regulated by DAO activity.[5]

Apart from this regulatory effect and polyamine-induced cell proliferation, DAO is an important enzyme in the gut mucosa for histamine (H) degradation.[8,9] In several tumor models alterations of tissue H content, reduced DAO activity, and increased polyamine synthesis have been mentioned.[1,5–7,10–13] This leads to the hypothesis that decreased DAO activity might be responsible for increased tissue polyamine and H concentrations.[5,12,14] This study compares DAO activity and H content in histologically normal gut mucosa (controls), nonneoplastic, and adenomatous tissue of patients with colorectal tubular/tubulovillous adenomas.

MATERIALS AND METHODS

In total, 245 colorectal samples from 15 control persons and 17 patients with tubular/tubulovillous adenomas were endoscopically taken from the lower gastrointestinal tract (terminal ileum to rectum) during diagnostic colonoscopy. After storage in liquid nitrogen, all samples were mechanically homogenized in phosphate buffer (5 minutes, 1500 rpm) using a microdismembrator (Braun Biotech, Germany), followed by centrifugation at 7500 rpm, 4°C, for 10 minutes. DAO

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activity (cpm/hr × mg wet weight [ww]; mean ± SD) was measured two hours after biopsy sampling using the [^{14}C]putrescine method described by Kusche *et al.*[15]

Tissue H content (ng/mg ww; mean ± SD) was determined in a 100-µl aliquot of the same sample that was added to 100 µl of perchloric acid in order to stop immediately enzymatic degradation of H. Measurement was performed after neutralization of the samples with KOH using a H radioimmunoassay[16] (Coulter-Immunotech, Germany).

For descriptive statistics mean ± standard deviation were used; statistical analyses were made by nonparametric Wilcoxon-test (U-test).

RESULTS

Mean DAO levels in the lower gastrointestinal tract were significantly different ($p = 0.0002$) between adenoma patients (65.8 ± 63, $n = 89$) and controls (93.4 ± 56, $n = 67$). In both groups, the regional distribution of DAO showed the highest activities in the terminal ileum, following a continuously decreasing gradient to the rectum. This result confirms the DAO distribution discovered by other groups that examined surgery specimens or only a few biopsies.[8,17] Mean DAO levels from cecum to the left colonic flexure were alike in adenoma (112.3 ± 39, $n = 32$) and control persons (117.4 ± 46, $n = 35$), whereas in all areas distal to the splenic flexure DAO levels of polyp patients were markedly diminished (61.2 ± 27, $n = 35$) in contrast to controls (98.4 ± 32, $n = 54$, FIG. 1).

The regional distribution of DAO in adenoma patients further revealed that the lowest DAO values in the rectum and colon sigmoideum were in inverse proportion to the numbers of polyps detected, whereas no adenoma occurred in the right hemicolon or areas showing DAO activities greater than 92.8 cpm/hr × mg ww. Furthermore, DAO activity 60, 30, 15, and 1–3 cm proximal to the adenoma amounted to 94.2 ± 22, 80.9 ± 71, 75.0 ± 65, and 48.6 ± 33, respectively ($n = 89$), whereas DAO was maximally decreased in adenoma tissue (47.06 ± 29, $n = 10$) or 1–3 cm distal of it (42.03 ± 55, $n = 15$).

The distribution of H in the right hemicolon was similar in both groups, but adenoma patients were characterized in all distal colonic segments by increased tissue H levels (FIG. 2).

DISCUSSION

Gut DAO is involved both in polyamine synthesis by deamination of P and in H catabolism,[9] two metabolic pathways not directly connected with each other. The results provide strong evidence for a decreased P degradation by DAO in adenoma patients, leading to high P concentrations that enhance polyamine synthesis.[1,2,5] Together with the impaired H catabolism by gut mucosal DAO,[8,14] resulting in significantly increased distal colonic tissue H levels in adenoma patients,[5,8,14] these findings suggest that DAO deficiency or impaired or inhibited

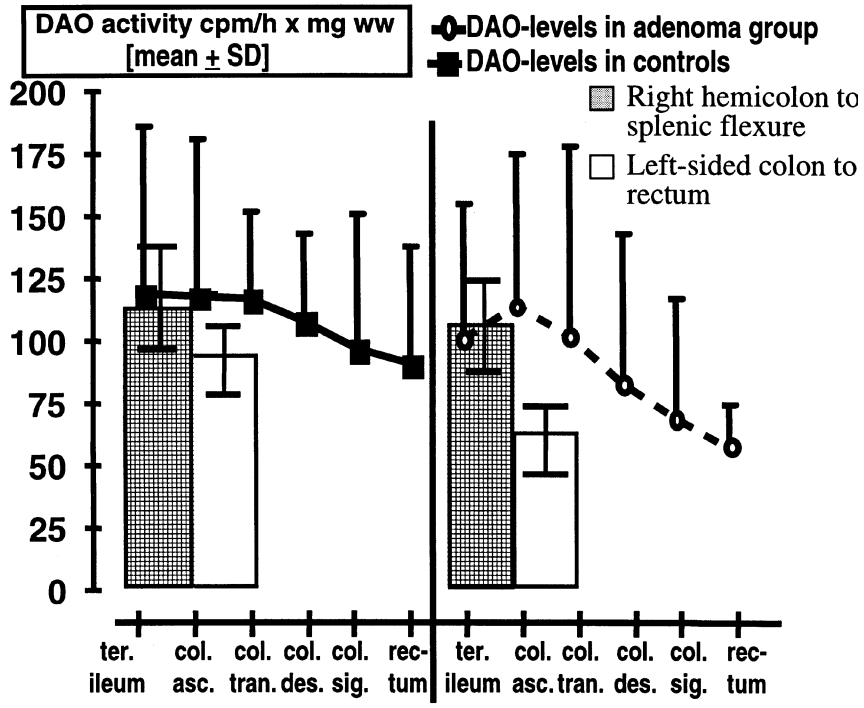

FIGURE 1. Regional distribution of DAO activity in the right hemicolon and left side of the colon of patients with colorectal adenoma and controls. Abbreviations: cpm, counts per minutes; term. ileum, terminal ileum; col. asc., colon ascendens; col. tran., colon transversum; col. des., colon descendens; col. sig., colon sigmoideum.

DAO activity in adenoma patients support the formation of highly mitogenic polyamines (spermidine, spermine).[2,5,10] In addition, H has also been reported to be mitogenic for human/intestinal epithelial cells.[13,18] Therefore, we conclude that the reduced DAO activity in adenoma patients synergistically enhances the mitogenic effects of locally accumulating polyamines and H, arising either from functional or constitutive disturbances of the DAO activitiy in these two functionally independent metabolic pathways.[1–3,5–7,10,14]

The occurrence of colorectal adenoma, particularly in areas with low DAO levels (left side of the colon), supports this hypothesis and demonstrates that the large bowel in adenoma patients is unable to produce DAO as a proliferation-terminating enzyme.[5,10] These findings possibly indicate a potential protective role of DAO as an antiproliferative enzyme in gut mucosa. These results clearly warrant further prospective studies using DAO as a biologic colonic tissue proliferation marker, which might enable an early identification of gut mucosal areas predisposed to or harboring adenoma.

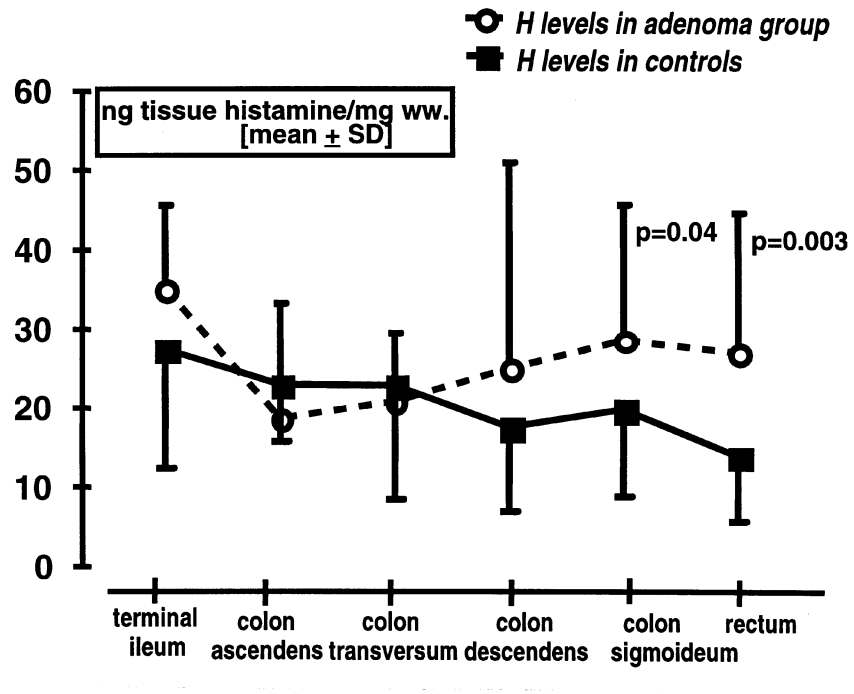

FIGURE 2. Regional distribution of histamine in the right hemicolon and left side of the colon of patients with colorectal adenoma and controls.

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