DNA Cytophotometry on Atypical Glands in Stomach Carcinogenesis of Dogs Induced by N-Methyl-N'-Nitro-N-Nitrosoguanidine

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Summary. Microspectrophotometric measurement of the DNA content of cell nuclei was performed on the lesions (including atypical glands) in gastric carcinogenesis of 15 male beagle dogs, which had been induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The distribution patterns of DNA content were classified into three types: normal, subnormal, and abnormal. The histograms of the distribution in normal and regenerative glands were a normal type and subnormal type, respectively, while adenocarcinoma showed an abnormal distribution type. In atypical glands, the distribution patterns in autopsy cases were subnormal and abnormal types. When sequential endoscopic observation of the angulus of the stomach in dog No.3 was carried out, atypical glands were found in an ulcer in the early stage of MNNG administration and a precancerous lesion in the late stage after termination of MNNG. The atypical glands in the early stage were of the subnormal type, while the atypical glands in the late stage were of the abnormal type. According to the results, these two types - subnormal and abnormal - of distribution of DNA content on the atypical glands may be related to regeneration and subsequent development of cancer, respectively.

Key Words: DNA cytophotometry – atypical glands – Dog stomach – MNNG

Introduction

Microspectrophotometric measurement of the DNA content of cell nuclei has been used in an attempt to illustrate the characteristics of cancer tissue objectively and quantitatively. In many studies, the distribution patterns of DNA content in malignant tumors were found to be different from those in benign tumors (Cuvelier and Roels 1979; Barlogie et al. 1980; Alho et al. 1983; Teodori et al. 1984; Kimura et al. 1984, Kreicbergs et al. 1984). The distribution patterns in the former showed an increase and wider scatter of DNA content, while the patterns in the latter showed a mode of DNA content in the diploid region and limited scatter up to the tetraploid region. Some authors reported that the measurement of DNA content made it possible to evaluate atypism in microscopic borderline lesions between malignancy and benignancy (Inui 1965; Hirose and Iwasa 1972). Other authors (Izuo et al. 1971; Barlogie et al. 1980) have reported that the lesions showing aneuploid distribution of DNA content develop into malignant tumors. For the reasons described above, the method used in these studies on the patterns of distribution can be recommended for detecting precancerous lesions in any organs. The possibility that so-called atypical epithelium or epithelial dysplasia is a borderline lesion between the benign state and malignancy in the human stomach has been studied (Nakamura et al. 1966; Nagayo 1971; Grundmann 1975; Morson et al. 1980; Nagayo 1981, Jass 1983), but its significance has not yet been elucidated. Atypical glands have been observed in carcinogenesis of the stomach in dogs induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). This observation, therefore, may be a useful model to study atypical epithelium in the human stomach. In the present study, serial observations on the lesions in the gastric carcinogenesis were endoscopically performed for more than 4 years (Saito et al. 1978), and atypical glands were divided into two types by the pattern of distribution in this study; one is a subnormal type which seemed to be related to regeneration and the other is an abnormal type which might have potential for developing malignant cancer.
Materials and Methods

Animals and Chemicals

Fifteen male beagle dogs (Kyudo Co., Kumamoto, Japan), weighing about 9 ± 1 kg, were given a solution of N-methyl-N' nitro-N-nitrosoguanidine (MNNG, Aldrich chemical Co., Inc. Milwaukee, Wis., USA) at the concentration of 50 or 83 mg/l with or without Tween 60 for 36-63 weeks, as described in the previous paper (Saito et al. 1978). Two beagle dogs without MNNG were used as the control group.

Histological Examination

The dog stomachs were periodically observed by endoscopy and biopsy. When the dogs died or were killed, necropsies were done carefully. The specimens obtained in biopsy or necropsy were fixed in a 10% formalin solution, embedded in paraffin, sectioned 5 μ in thickness, stained regularly with hematoxylin and eosin (H & E) and PAS-AB staining, if necessary, and a histopathological examination was performed. Atypical glands were determined by showing an intermediate degree of structural and cellular atypicality between regenerative glands and adenocarcinoma. The atypical glands were differentiated carefully from inflammatory or regenerative changes associated with active inflammation and from malignant change.

DNA Staining

Feulgen staining was done to sections 10 μ in thickness according to the modified Koana-Naora's method (Naora 1955).

After tissue was first histologically examined on H&E sections, the adjacent sections were deparaffinized, dehydrated in a 1N HCl solution at 60 °C for 4 min, incubated in Schiff's reagent for 3 h, and rinsed 3 times in an aqueous sulfate for 15 min. Then the sections were rinsed in tap water for 5 min, dehydrated in alcohol, and covered with a Canada-Valasam coverglass.

Measurement of DNA Contents

DNA contents of cell nuclei were measured by microspectrophotometer (MMS, Olympus Co., Tokyo, Japan) according to the Patau's two-wave length method (Patau 1952) using Mendelsohn's calculating table (Mendelsohn 1958). Two waves giving the maximum absorption index (2a) and half of the maximum (2b) were determined in each preparation by drawing an absorption curve for lymphocytes in the tissue. The range of waves used was 495-505 μm for 2a and 555-560 μm for 2b. Cell nuclei (50-100) were measured at random in each lesion. Measurement was confined to the cells that kept the complete form of the nuclei and did not overlap. The diploid value (2c) was determined by the mean value of Feulgen DNA contents of 25-50 stromal lymphocytes in each tissue. DNA content was illustrated by an arbitrary unit, which was the relative amount of DNA to 2c. DNA content was illustrated by an arbitrary unit.

The Pattern of the Distribution of DNA Content

The patterns in the histogram were divided into three types: normal, subnormal, and abnormal distribution types. In the normal type (Fig. 1 a), the average DNA content was diploid and the distribution was unimodal with a high and narrow mode. Some tetraploid value occurred. In the subnormal type (Fig. 1 b), the average DNA content was diploid, hyperdiploid, or hypodiploid and the distribution was unimodal with a considerable high and broad mode. Some tetraploid or semitetraploid values occurred. In the abnormal distribution type (Fig. 1 c), three subtypes of the distribution were seen: (1) a bimodal distribution which had both the primary mode in hyperdiploid or hypodiploid and the secondary mode in tetraploid range (Fig. 2-1), (2) a broad and unimodal distribution which had the modal DNA value of tetraploid range (Fig. 2-2) and (3) a broad and aneuploid distribution which had multiple modes or no prominent mode (Fig. 2-3). The scatter of cell nuclei extended up to 8 c and DNA content above 4 c was over 12% in each subtype.