Effects of Acetylsalicylic Acid and Celecoxib on the N-Nitrosodiethylamine Induced Carcinogenesis in Rat Liver and Esophagus

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The effects of nonsteroid antiinflammatory drugs (acetylsalicylic acid and celecoxib) on N-nitrosodiethylamine-induced carcinogenesis in the liver and esophagus were studied in rats. The inhibitory effect of celecoxib on carcinogenesis was more pronounced (in comparison with acetylsalicylic acid), which manifested in a significantly decreased incidence of neoplastic changes in the liver tissue (from 91.7 to 65.2%), number of tumors in the esophagus (from 4.13 to 2.61 tumor/rat), and in delayed malignization in the liver and esophagus. The incidence of erosions and ulcers of the gastric mucosa was significantly lower after celecoxib treatment. These data indicate that celecoxib inhibits N-nitrosodiethylamine-induced carcinogenesis in the liver and esophagus.

Key Words: *liver and esophagus carcinogenesis; N-nitrosodiethylamine; drug prevention; acetylsalicylic acid; celecoxib*

Chemoprophylaxis is an independent perspective trend in the prevention of malignant diseases. Its aim is identification and introduction into clinical practice of bioactive substances with anticarcinogenic effect, which can inhibit carcinogenesis at different stages. Nonsteroid antiinflammatory drugs (NSAIDs) attract special attention among the few agents with proven anticarcinogenic effect. However, the overwhelming majority of studies were focused on the protective role of NSAIDs for colorectal cancer. Based on the results of these studies, celecoxib, a selective inhibitor of cycloxygenase-2 (COX-2) was approved by the Foodstuff and Drug Administration (FDA) of the USA as a means for chemical prevention of colorectal cancer in families with adenomatous polyposis. Solitary studies carried out with malignant tumors of other location do not permit final conclusions [6].

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We studied the effects of acetylsalicylic acid and celecoxib on N-nitrosodiethylamine (NDEA) induced carcinogenesis in rat liver and esophagus.

MATERIALS AND METHODS

Experiments were carried out on 80 male Wistar rats (initial weight 100-120 g) from the vivarium of Pyatigorsk State Pharmaceutical Academy. The animals were kept 5-6 per cage under standard conditions at 20-22°C and natural day/night regimen on standard vivarium ration with free access to water.

Liver and esophageal tumors were induced as described previously [2]: NDEA in a concentration of 100 mg/liter was added into drinking water during 4 months. The animals were divided into 3 groups: group 1 (n=30) animals received the carcinogen alone (control); group 2 (n=25) received, in addition to the carcinogen, acetylsalicylic acid (nonselective inhibitor of cycloxygenase, COX; Rosmedpreparaty Company; 400 mg/kg fodder); and group 3 (n=25) animals received celecoxib (cele-

brex), a highly selective inhibitor of COX-2 (SEARL; 1500 mg/kg fodder). The doses were selected in accordance with published data: acetylsalicylic acid and celecoxib in these doses exhibited the maximum anticarcinogenic activity on models of colorectal, hepatic, urinary bladder, and breast cancer [4,8,12,14]. The drugs were mixed with custard and added to the rations of experimental groups from the first day of NDEA challenge until the end of the experiment lasting for 38 weeks. The animals completely ate the fodder containing the studied substances. Rats surviving until the end of the experiment were sacrificed by fluothane vapor. At autopsy the liver was evaluated macroscopically by the following arbitrary score [1]: 0: no changes; 1: heterogeneous color at some sites, no focal changes; 2: solitary tumor nodules 0.1-0.2 cm in diameter; 3: multiple 0.1-0.2-cm nodules or solitary 0.3-0.5-cm nodules; 4: multiple 0.3-0.5 nodules or solitary 0.6-1.0-cm nodes; 5: multiple 0.6-1.0-cm nodes or solitary nodes larger than 1 cm in diameter; 6: multiple nodes larger than 1 cm in diameter; 7: tumors larger than 3 cm in diameter, tumor tissue predominating. All tumors in the esophagus were counted and measured, the multiplicity index was calculated with regard to the number of rats in the group. Material collected for histological study was fixed in 10% formalin, embedded in paraffin, and the sections were stained by hematoxylin and eosin. Analysis of microscopic changes in the liver and esophagus was based on classification of tumors in laboratory animals, suggested by International Agency for Research of Cancer [11]. Microscopic evaluation of neoplastic changes was carried out using a 3-point scale [2]: 1 point: early neoplastic changes; 2 points: benign tumors; 3 points: malignant tumors.

The efficiency of the modifying effect on hepatic and esophageal carcinogenesis was evaluated by changes in the number of rats with neoplastic processes, index of neoplasm multiplicity, mean score of macro- and microscopic changes in experimental animals in comparison with controls.

The results were statistically processed. The significance of difference was evaluated using Student's t, Mann—Whitney and χ^2 tests.

RESULTS

Neoplastic changes were detected in 22 control group animals (91.7% of effective number; Table 1). The first rat with precancer changes in the liver died during week 11 of the experiment. Microscopic examination of the liver tissue showed ductal hyperplasia and foci of hepatocyte eosinophilic and basophilic cell hyperplasia. Malignant tumors (well-differentiated hepatocellular cancer and cholangiocellular cancer, in both cases developing in the presence of cirrhosis and hyperplasia of the ducts and/or hepatocytes) under experimental conditions were first identified in 2 rats dead during week 22. Hence, manifestations of tumor progress in the liver tissue were observed. Morphological analysis of neoplastic changes identified well-differentiated, moderately, or poorly differentiated hepatocellular cancer in 13 control rats, cholangiocellular cancer in 1, hepatocellular adenoma in 2, and cholangiofibrosis in 1 rat. These processes developed against the background of diffuse and focal hyperplasia of hepatocytes and/or ducts and cirrhosis of different severity.

Neoplastic changes were detected in 16 (76.2%) rats treated with acetylsalicylic acid. Microscopic analysis of histological material in this group showed hepatocellular cancer in 4 cases, hepatocellular adenoma in 2, and cholangioma in 1 rat. Diffuse or focal hyperplasias of hepatocytes and/or ducts were detected in 15 cases, with cystic hyperplasia of the ducts in 3 of these cases.

A total of 65.2% rats developed neoplastic changes in the group treated with celecoxib ($p<0.05$; Table 1). Histological study detected 2 cases with cholangiofibrosis and 2 with dysplasia, 14 with ductal hyperplasia (including cystic) and/or hepatocyte hyperplasia. A statistically significant reduction of the mean score of macro- and microscopic changes in liver tissue was noted (Table 1).

Acetylsalicylic acid or celecoxib added to the ration of animals receiving NDEA virtually did not change the incidence of tumor development in the esophagus (Table 2). Celecoxib treatment 1.6-fold reduced the multiplicity index. The mean score of microscopic changes in the esophagus decreased 1.2 times under the effect of acetylsalicylic acid and 1.4 times in comparison with the control under the effect of celecoxib.

The study of side effects of these NSAIDs showed deep erosive ulcerative lesions in the gastric mucosa in 78.3% rats treated with acetylsalicylic acid. In the celecoxib group, surface erosions of the gastric mucosa were detected in 17.4% rats.

Hence, in our experiment the effects of acetylsalicylic acid and celecoxib on hepatic and esophageal carcinogenesis manifested in delayed malignization. We used acetylsalicylic acid in a high dose corresponding to 80% of the maximum tolerable dose under conditions of long treatment, which contradicts the opinion of many scientists advocating good prospects of low dose use of this drug for simultaneous prevention of cardiovascular diseases and malignant tumors [12-14]. Anticar-

TABLE 1. Effects of Acetylsalicylic Acid and Celecoxib on NDEA-Induced Carcinogenesis in Rat Liver

Group	Effective number of rats in group	Number of rats with neoplastic changes		Macroscopic evaluation, score	Microscopic evaluation, score
		abs.	%		
Control	24	22	91.7	2.67±0.34	2.17±0.25
Experimental					
1 (acetylsalicylic acid)	21	16	76.2	1.24±0.22**	1.29±0.24*
2 (celecoxib)	23	15	65.2*	0.65±0.11***	0.87±0.15***

Note. *p<0.05, **p<0.01, ***p<0.001 compared to the control.

TABLE 2. Effects of Acetylsalicylic Acid and Celecoxib on NDEA-Induced Carcinogenesis in the Rat Esophagus

Group	Effective number of rats in group	Number of rats with neoplastic changes		Multiplicity index	Microscopic evaluation, score
		abs.	%		
Control	23	21	91.3	4.13±0.35	2.13±0.11
Experimental					
1 (acetylsalicylic acid)	19	16	84.2	4.11±0.51	1.79±0.11*
2 (celecoxib)	23	17	73.9	2.61±0.44**	1.57±0.15*

Note. *p<0.05, **p<0.01 compared to the control.

cinogenic effect of celecoxib was more pronounced in comparison with acetylsalicylic acid. Treatment with this drug led to a significant reduction of the incidence of neoplastic changes in the hepatic tissue, in the number of esophageal tumors, and delayed malignization in the liver and esophagus. Celecoxib exhibited a more pronounced (in comparison with acetylsalicylic acid) anticarcinogenic activity on the model of experimental carcinogenesis, with a significantly lower incidence and severity of lesions in the gastric mucosa.

The mechanisms underlying the anticarcinogenic activity of NSAIDs are little studied. It is known that the main mechanism of antiinflammatory and analgesic effects of these drugs is inhibition of COX and hence, blockade of prostaglandin synthesis from arachidonic acid. Presumably, this mechanism is the leading one in the realization of anticarcinogenic activity of NSAIDs. The key role here belongs to activity of drugs towards COX-2 isoenzyme (whose hyperexpression detected in malignant tumors of different location and was linked with intensification of cell proliferation and angiogenesis, suppression of tumor cell apoptosis, and immune response suppression [3,6]). In addition, COX-2 selective inhibitors can modify the carcinogenesis process by modulating the COX-independent mechanisms: antimutagenic activity of celecoxib, its capacity to suppress LPO, and reduce the expression of proliferation markers were shown [5,10].

One of the main requirements to chemoprophylactic drugs is their safety in long-term treatment. The use of nonselective inhibitors of COX (the majority of standard NSAIDs) for preventive purposes is limited by high incidence of side effects, primarily involvement of the gastrointestinal mucosa. Great expectations are therefore connected with a new class of drugs, selective COX-2 inhibitors, *e.g.* celecoxib. It is expected that it will provide a pronounced antitumor effect at a lower risk of serious side effects, because long-term treatment with selective COX-2 inhibitors is associated with 50-60% lesser incidence of serious gastrointestinal side effects than the use of traditional NSAIDs [9]. However, side effects for the cardiovascular system are discussed, such as the risk of thromboembolic complications, myocardial infarction, and arterial hypertension, particularly in elderly subjects. Dose-dependent development of these complications was detected and the best safety profile of celecoxib in comparison with all the rest currently used highly selective inhibitors of COX-2 was shown, on condition that it is used in the minimum effective dose during a limited period [5,7,9].

Hence, COX-2 selective inhibitors, similarly as traditional NSAIDs, cannot be considered as "universal" preventive drugs. However, there are serious prerequisites for further studies of their anticarcinogenic activity and use of highly selective inhibitors of Cox-2 as drugs for "target drug prevention" of malignant tumors in high risk groups.

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