Depression of experimental gastric ulcers and acute pancreatitis in rats treated by 5-azacytosine, 5-azacytidine and their N-methyl derivatives

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Summary. 5-Azacytosine, 1-methyl-5-azacytosine and 5-azacytidine administered to rats with a ligated pylorus block gastric secretion, gastric acidity, the extent of hemorrhage and the number and size of gastric defects. The same drugs also depress the development of experimental acute pancreatitis mediated in rats by interstitial administration of 7.5% natrium cholate into the pancreas in vivo. The drugs affected the amount of abdominal fluid and 6 h after the treatment the pathological changes were significantly decreased.

Key words. 5-Azacytosine; 5-azacytidine; rat pancreatitis; gastric secretion.

During the study of side effects of 5-azacytidine used for the treatment of acute leukemia, gastrointestinal disturbances were often observed[1,2]. 5-Azacytidine was later shown to block gastric secretion, in association with a 2–3-fold increase of the stomach weight[3,4], and to prevent the development of experimental pancreatitis in rats[5]. In this paper we followed the effect of different N-methyl derivatives of 5-azacytosine and 5-azacytidine on the incidence of gastric ulcers and on the development of acute pancreatitis in rats. While 5-azacytidine is incorporated[6,7] into nucleic acids the incorporation of 5-azacytosine and of its N-alkyl derivatives has not been demonstrated.

Materials and methods. 5-Azacytidine, 5-azacytosine and their N-methyl derivatives were prepared as described[8]. Male Wistar rats (180–250 g) kept under standard conditions were fasted 18 h prior to ligation. Pyloric ligation[9] was performed at 14.00 h; groups of 8–11 animals received the drugs i.p. (5–30 mg/kg) and were killed 22 h later. Gastric juice was collected and analyzed as described earlier[3,4]. The classification system[3] applied for the evaluation of gastric ulcers takes into consideration the number and the size of gastric defects as well as the extent of the hemorrhage.

In case of experimental pancreatitis laparotomy was carried out under mild ether narcosis and 0.6 ml 7.5% natrium cholate was administered interstitially into the pancreas[10]. The tested drugs were administered s.c. in a maximal volume 0.3 ml. 6 h later the animals were killed by cervical dislocation and the volume of abdominal fluid was measured. The macroscopic changes in the pancreas and abdominal cavity were evaluated as described[5]. The highest number of points was allocated to hemorrhagic and necrotic changes, followed by necrosis of the pancreas, and edema and necrosis in the abdominal cavity. The data were analyzed for statistical significance by Student's t-test.

Results and discussion. 5-Azacytidine at relatively low doses blocks gastric secretion[3,4]. This effect is accompanied by decreased secretion of pepsin and especially by lowered gastric acidity[4]. Unfortunately 5-azacytidine is toxic and has undesired side effects that do not permit its use as an antiulcer drug. In an attempt to decrease the toxicity of 5-azacytidine different derivatives of 5-azacytosine were prepared and their antisecretory activity was measured. The incorporation of 5-azacytosine and its derivatives into rat liver nucleic acids could not be demonstrated although the biological action and toxicity of 5-azacytidine are generally associated with its incorporation[11–13].

The data presented in table 1 show the effect of various triazines on gastric secretion, gastric acidity and the formation of gastric ulcers. Besides 5-azacytidine, 5-azacytosine and 1-methyl-5-azacytosine also display significant antisecretory activity. Other tested N- and O-substituted derivatives of 5-azacytosine were without activity in this respect.

The decrease of gastric secretion and the depression of the formation of gastric ulcers in relation to the dose of i.p. administered 5-azacytosine are shown in the figure. In the case of 1-

Table 1. Effect of 5-azacytosine and its derivatives on gastric secretion in rats

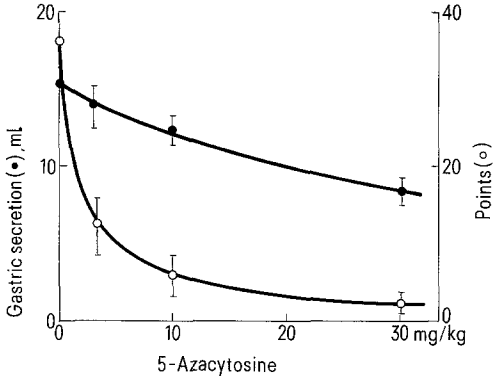
Compound (30 mg/kg)	No. of points ± SE	Gastric secretory volume (ml ± SE)	Gastric acidity, H^+ (mmol ± SE)
Control	27.4 ± 4.1	17.4 ± 1.2	1.28 ± 0.14
5-Azacytosine	3.1 ± 1.7^{++}	7.1 ± 0.8^{+}	0.38 ± 0.10^{+}
0-Methyl-5-azacytosine	32.7 ± 6.3	14.7 ± 2.6	1.20 ± 0.26
1-Methyl-5-azacytosine	4.1 ± 1.4^{++}	9.5 ± 1.2^{+}	0.34 ± 0.06^{+}
6-Ethyl-5-azacytosine	27.3 ± 6.9	15.0 ± 2.6	1.07 ± 0.21
1,4′-Dimethyl-5-azacytosine	25.5 ± 6.0	18.0 ± 1.2	1.21 ± 0.07
4′,4′,6-Trimethyl-5-azacytosine	25.0 ± 6.1	21.3 ± 1.3	1.30 ± 0.06
5-Azacytidine	3.8 ± 1.7^{++}	3.1 ± 0.8^{++}	0.11 ± 0.01^{++}
1-β-D-Glucopyranosyl-5-azacytosine	21.5 ± 6.5	15.9 ± 0.9	0.96 ± 0.04

Groups of 8–11 animals (200 g) starved 18 h were subjected to pyloric ligation and immediately injected i.p. with the drugs. 22 h later they were killed, gastric defects evaluated (points) and gastric juice collected and analyzed. $^{+}p < 0.05$; $^{++}p < 0.01$.

Table 2. Development of experimental acute pancreatitis in rats treated by N-methyl derivatives of 5-azacytosine and 5-azacytidine

Compound (mg/kg)	No. of rats	No. of points ± SE	Abdominal fluid (ml ± SE)
Control	11	25.0 ± 1.2	4.44 ± 0.68
5-Azacytosine (30)	10	14.4 ± 0.8^{+}	3.60 ± 0.92
1-Methyl-5-azacytosine (30)	8	15.3 ± 2.1^{+}	3.63 ± 0.63
1,4′-Dimethyl-5-azacytosine (30)	9	23.1 ± 2.6	4.78 ± 0.28
4′,4′,6-Trimethyl-5-azacytosine (30)	8	23.7 ± 2.7	5.50 ± 0.48
5-Azacytidine (5)	10	9.0 ± 1.5^{+}	2.68 ± 0.57^{+}
4-Methyl-5-azacytidine (15)	8	18.0 ± 1.1	3.20 ± 0.17
4′,4′-Dimethyl-5-azacytidine (15)	8	20.6 ± 1.9	3.42 ± 0.42

The animals (250 g) were killed 6 h after the s.c. administration of the drugs and macroscopic changes (points) and volume of abdominal fluid were measured. $^{+}p < 0.05$.



Depression of gastric ulcers and gastric secretion in rats by 5-azacytosine. Groups of 10 male animals received the drug i.p. immediately after pyloric ligation and 22 h later they were killed by cervical dislocation.

methyl-5-azacytosine the increase of its dosage to 50 or 100 mg/kg results in the inhibition of gastric secretion and gastric acidity but the action on the process of ulceration is less favorable. Hydrolytic products of 1-methyl-5-azacytosine and 5-azacytosine, N-amidino-N'-methyl urea and N-amidine methyl urea, respectively, are completely inactive.

In an earlier study we observed depression of experimental acute pancreatitis mediated in rats by 5% bile solution administered into the pancreas in vivo following 5-azacytidine or cycloheximide treatment[5]. Here we show that 5-azacytosine and its 1-methyl derivative also decrease the amount of abdominal fluid and prevent pathological changes in the pancreas (table 2) 6 h after the interstitial administration of natrium cholate. Other tested methyl derivatives of 5-azacytosine and 5-azacytidine were inactive in this respect.

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Specific determination of arylsulfatase A activity

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Summary. Arylsulfatase activities in biological materials are too low to be detected by the methods available hitherto. A sensitive and specific assay method for arylsulfatase A (AS-A) has been developed in the present study. Ascorbate-2-sulfate is known to be a specific natural substrate of AS-A; the ascorbic acid liberated by the action of AS-A was quantitatively determined using HPLC equipped with an amperometric detector. The method was used to analyze the activity of AS-A in biological materials.
Key words. Arylsulfatase A; natural substrate; ascorbate-2-sulfate; HPLC-amperometric detection.

Arylsulfatase [EC 3.1.6.1 arylsulfate sulfohydrolase] (AS) activities have been shown to be present in biological materials in connection with diseases. Three types of AS activities are known at present in mammalian tissues; AS-A, AS-B and AS-C. AS-A and AS-B are present in the lysosomal fraction of the cell. Since changes in AS-A and AS-B activities have been shown in disease states[1,2], AS activity determination could be useful in characterizing the nature of some diseases. For example, it has been documented that AS-A activity is absent in metachromatic leukodystrophy (MLD) and AS-B is absent in the Maroteaux-Lamy syndrome (MLS)[1,2].

Human leukocyte and rat brain arylsulfatase A activity with the natural substrate. HPLC conditions and assay procedure for arylsulfatase A activity; same as in the chart

Arylsulfatase A activity	Mean ± SD	Number
Human leukocytes	2.39 ± 0.90[a]	N = 8
Rat brain	112.90 ± 69.10[b]	N = 5

[a] Ascorbic acid nmoles/30 min/mg protein; [b] ascorbic acid nmoles/h/mg protein.

For the purpose of discriminative determination of AS-A and AS-B activities, specific inhibitors for each activity have been proposed[3,4]. However, the inhibitory effects of these compounds are not strictly specific. The enzyme activity was found to be variable among different animal species. Therefore, a universally applicable method would be valuable, but such a method has not been found so far.

There are natural substrates for AS-A and AS-B activities, and the enzyme activities are more specific for the natural substrates than for synthetic ones. UDP-N-Ac-galactosamine-4-sulfate is known to be a good and specific substrate for AS-B[5] and ascorbate-2-sulfate (AAS) for AS-A[6].

In the present investigation, AS-A activity was specifically assayed using AAS; this excludes the possibility of AS-B activity. The enzymatic reaction product, ascorbic acid (AA), could be separated from the unreacted substrate, sulfate and enzyme by HPLC.

Materials and methods. All chemicals used were of analytical grade or reagent grade, and obtained from Sigma Chem. Co., St. Louis, MO (USA) and from Wako Pure Chem. Co., Tokyo (Japan). HPLC column is LiChrosorb RP-18, 7 μm, of Merck (FRG).