Nuclear DNA Analysis of the Periampullary Carcinoma Using Cytologic Bile Specimens

KENJI KAKIZAKI and HIDEMI YAMAUCHI

Department of Surgery, Sendai National Hospital, Sendai

Analysis of nuclear DNA seemed to be a reliable tool to evaluate the biological aggressiveness of pancreatic and periampullary carcinomas (Yamauchi et al. 1989) using tissue samples obtained operatively. However, no studies of DNA analysis of periampullary carcinoma have been performed preoperatively, because it is hard to obtain sufficient specimens for DNA analysis. We tried the DNA analysis of the carcinoma cells collected from the bile specimen by using a cytofluorometric method (Takahama and Kagaya 1988). Briefly, the cell suspension obtained from a bile sample through the percutaneous biliary drainage tube was smeared after staining with diamidino-phenylindole and fluorescence intensity of 100 carcinoma cells for each case was measured with a spectrophotometric microscope. The DNA content of 20 lymphocytes per case served as a reference to establish the 2c level.

The DNA histograms and cytologic findings are shown in Fig. 1. Clinical data of the patients were as follows. Patient 1 was a 39-year old male with adenosquamous carcinoma of the pancreas with multiple liver metastases. A bypass operation was carried out on the patient but he died of the primary disease three months after the operation. The DNA ploidy pattern was aneuploid and polyploid. The DNA content was 4.8±1.8 c (mean±s.d.), which was higher than those of the other two cases. Patient 2 was a 62-year old female with well differentiated adenocarcinoma of the common bile duct. Curative resection was performed. DNA histograms of this case showed a near diploid pattern with a peak in the 2c range. The DNA content was 2.8±1.0 c, which was the lowest in the three cases. Patient 3 was a 76 year-old female who presented obstructive jaundice and died in three months after onset of jaundice. The cytologic examination of bile showed adenocarcinoma, which was aneuploid and had the DNA value of 3.8±1.3 c.

Received April 19, 1991; revision accepted for publication October 10, 1991.

This work was supported in part by the Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare of Japan.
The DNA histograms using bile sample could be evaluated as well as those using paraffin embedded tissue samples and it is likely that biological behavior of periampullary carcinoma might be estimated preoperatively by DNA analysis of carcinoma cells obtained from bile specimens.

References


Short Report

Potent Vasoconstriction by Tyramine but No Significant Constriction by Nicotine in Isolated Dog Ear Arteries

SHIGETOSHI CHIBA and MIYOKO TSUKADA

Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390

CHIBA, S. and TSUKADA, M. Potent Vasoconstriction by Tyramine but No Significant Constriction by Nicotine in Isolated Dog Ear Arteries. Tohoku J. Exp. Med., 1991, 165 (3), 239-241 — Using the cannula inserted method, vascular effects of nicotine were investigated in isolated, perfused dog intermediate auricular arteries, comparing with those of norepinephrine and tyramine. Norepinephrine and tyramine produced strong vasoconstrictions in a dose-related manner, but nicotine did not induce any significant vasoconstriction. —— cannula inserted method; dog ear artery; nicotine; tyramine

Previously Chiba and Ito (1985) reported that tyramine induced strong vasoconstrictions in isolated and perfused dog intermediate auricular arteries, using the cannula inserted method which was developed by Hongo and Chiba (1983). They also reported that this preparation responded well to periarterial electrical nerve stimulation (Chiba and Ito 1985). It is well known that tyramine readily produces indirect sympathomimetic effect mediated via catecholamine release from sympathetic nerve terminals by the uptake mechanism. On the other hand, nicotine is also recognized to cause a catecholamine release by a different mode of action from that of tyramine (Kirpekar et al. 1980). Nicotine causes a release of norepinephrine from postganglionic sympathetic nerve terminals by activating prejunctional nicotinic receptors (Richardson and Woods 1959; Bhagat et al. 1968; Lindmar et al. 1968). In the heart, either a bolus injection of tyramine or nicotine readily caused a release of norepinephrine, which in turn produced positive chrono- and inotropic effects (Chiba et al. 1972; Ren et al. 1991). Thus, in the present study, we made an attempt to evaluate vascular responses to nicotine comparing with those to tyramine and norepinephrine.

Mongrel dogs of either sex (7-12 kg) were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After treatment with sodium heparin (200 units/kg, i.v.), the animals were sacrificed by rapid exsanguination from the right common carotid artery. The intermediate auricular (ear) artery of either ear was then carefully isolated, and 1-3 segments (without large branches, 0.4-0.7 mm in outer diameter, 5-8 mm in length) were cut from each isolated artery. The segments were cannulated and set up for perfusion with a constant flow rate. The perfusate contained 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgCl2, 2.5 mM CaCl2, 25 mM NaHCO3 and 5.6 mM glucose and was babbled with mixture of 95% O2 and 5% CO2 and maintained at a constant temperature of 37°C. The rate (1-2 ml/min) was adjusted at the beginning of the experiments to obtain a control perfusion pressure of approximately 100 mmHg. The perfusion pressure was measured with an electronic manometer, and the vasoconstriction induced by intraluminal administration of a drug was

Received August 19, 1991; revision accepted for publication October 14, 1991.
recorded as an increase in perfusion pressure. Drugs used were tyramine hydrochloride (Tokyo Kasei, Tokyo), norepinephrine hydrochloride (Sankyo, Tokyo) and L-nicotine bi-d-tartrate (Tokyo Kasei, Tokyo). They were dissolved in saline. Each drug solution was intraluminally administered into the perfusion line close to the cannula in a volume of 0.01-0.03 ml over 4 sec by use of a microinjector. The experiments were started when the arteries had equilibrated for approximately 1 h until perfusion pressure became stable.

When tyramine or norepinephrine was intraluminally injected to the isolated dog intermediate auricular artery, vasoconstriction was induced in a dose-related manner as previously reported (Ito and Chiba 1984; Chiba and Ito 1985). The threshold doses for norepinephrine and tyramine were 0.01 and 0.3 μg, respectively, and their maximum increases in perfusion pressure were approximately 200 mmHg. On the contrary, nicotine did not produce significant changes in perfusion pressure, indicating that an intraluminal bolus injection of nicotine has no significant vascular action. Fig. 1 shows the dose-response curves for norepinephrine, tyramine and nicotine.

Recently, Ren et al. (1991) demonstrated that nicotine induced markedly positive inotropic effects in both isolated atrial and ventricular preparations of dog hearts in almost the same dosage. It has been well known that nicotine acts on nicotinic receptors of the adrenergic nerve terminals and releases norepinephrine from these terminals (Muscholl 1970). However, in the present study nicotine failed to produce any significant vasoconstriction. Since tyramine showed a strong vasoconstriction, there might be densely nerve terminals as reported previously (Chiba and Ito 1985). Although the reason why intraluminal administration of nicotine has no significant effect on isolated, perfused intermediate auricular arteries is unclear, it is considered that 1) a bolus injection of nicotine may have difficulty to reach sympathetic nerve terminals (adventitia area of the vessel) from the endothelial side in the dog blood vessel, and 2) vascular vessels show extensive regional and species variations in the density of various receptors, sympathetic innervation and morphological arrangements. Bell (1968) reported using the perfused rabbit ear artery preparation that injection of nicotine intraluminally caused vasoconstriction, and he assumed that this constriction was due to neuronal norepinephrine release. In the perfused isolated central ear artery of the rabbit, a relatively high concentration of nicotine produced transient vasoconstriction (Steinsland and Furchgott 1975a). However, Rand and Varma (1970) and Hume et al. (1972) did not obtain a constrictor response to ACh at high concentrations. Steinsland and Furchgott (1975b) considered that Rand and Varma (1970) and Hume et al. (1972) used graded cumulative additions of the agonist-starting with a low concentration below the threshold concentration for stimulation and
then cumulatively increasing the concentration until a high concentration was reached. If this was indeed the procedure used by these workers, then cumulative desensitization of the receptors, beginning at subthreshold concentrations of agents, could have prevented any responses with successive increasing in concentrations. In the present study, it is difficult to consider such cumulative desensitization, because we had adequate time intervals between drug injections.

Although we have no explanation to fail any vascular response to nicotine, it is necessary to investigate regionally different preparations and species differences in this method in future.

References