The effects of niacin, namely, nicotinic acid and nicotinamide, and trigonelline on the proliferation and invasion of cancer cells were studied using a rat ascites hepatoma cell line of AH109A in culture. Niacin and trigonelline inhibited the invasion of hepatoma cells at concentrations of 2.5–40 μM without affecting proliferation. Hepatoma cells previously cultured with a reactive oxygen species (ROS)-generating system showed increased invasive activity. Niacin and trigonelline suppressed this ROS-potentiated invasive capacity through simultaneous treatment of AH109A cells with the ROS-generating system. The present study indicates for the first time the anti-invasive activities of niacin and trigonelline against cancer cells.

Key words: hepatoma; invasion; nicotinamide; nicotinic acid; trigonelline

Endless proliferation and metastasis are two biological properties of cancer cells. Metastasis is the primary cause of death in human cancer. Cancer metastasis is attained by a sequence of steps, of which invasion is a particularly complicated process and the key step in the metastatic cascade. Furthermore, invasion and metastasis might be correlated with reactive oxygen species (ROS). Some food factors with antioxidative activity inhibit the invasion of hepatoma cells. Coffee is reported to suppress tumor cell invasion by reducing oxidative stresses in vitro and ex vivo. Trigonelline is a major constituent in coffee and niacin-related compound. Niacin is one of the water-soluble vitamins, and reportedly has moderate radical-scavenging activity. The present study was attempted to define the effects of niacin, i.e., nicotinic acid and nicotinamide, and trigonelline on the proliferation and invasion of hepatoma cells.

Nicotinic acid and nicotinamide were purchased from Tokyo Kasei Kogyo (Tokyo), and trigonelline from Sigma Chemical (St. Louis, MO). Niacin and trigonelline were dissolved in culture medium and sterilized with filtration prior to use.

A rat ascites hepatoma cell line of AH109A was provided by the Institute of Development, Aging, and Cancer of Tohoku University, Sendai, Japan. AH109A cells were maintained in the peritoneal cavities of male Dorny rats, prepared from accumulated ascites, and cultured in vitro in Eagle’s minimum essential medium (MEM, Nissui Pharmaceutical, Tokyo) containing 10% calf serum (CS, obtained from Gibco BRL, Grand Island, NY) (10% CS/MEM), as described previously. To eliminate contaminated macrophages and neutrophils, cells were cultured at least for 1 week after isolation and used for the assays described below.

The effects of niacin and trigonelline on the proliferation of AH109A cells were examined by measuring the incorporation of [methyl-3H]thymidine (20 Ci/mmol, New England Nuclear, Boston, MA) into the acid-insoluble fraction of cells for 4 h, as described previously. The effects of niacin and trigonelline on the invasion of AH109A cells were examined by the co-culture system, with slight modifications as described previously. Briefly, mesothelial cells (M-cells) were isolated from the mesentery of male Dorny rats (6–8 weeks old). After digestion by trypsin, 1.2 × 10^5 cells were plated in a 60-mm culture dish with 2-mm grids (Nunc A/S, Roskilde, Denmark), and cultured for 7–10 days to attain a confluent state in 10% CS/MEM. Then AH109A cells (2.4 × 10^5 cells per dish) were applied on the monolayer of M-cells in 10% CS/MEM with niacin or trigonelline for 24 h and 48 h respectively. Invaded cells and colonies underneath M-cells were counted with a phase-contrast microscope. Usually ten areas were counted, and the invasive activity of AH109A cells was expressed as the number of invaded cells and colonies/cm^2.

To examine the bioavailability of trigonelline, the time-dependent effect on the proliferation and invasion of AH109A was studied using trigonelline-loaded rat serum. Trigonelline was suspended in a 0.3% carboxymethyl cellulose sodium salt (CMC, Wako Pure Chemicals, Osaka, Japan) aqueous solution at a concentration of 17.4 mg (= 100 μmol)/ml. Trigonelline suspension was intubated at a dose of 100 μmol/ml/100 g body weight to male Dorny rats (6 weeks old) which had been fasted overnight, and blood was collected at the time points indicated in Fig. 2B. Serum was prepared by centrifugation, sterilized by filtration, and added to the culture media at a concentration of 10% instead of CS for the proliferation and invasion assays. The prolifer-
ative and invasive activities of AH109A in the presence of trigonelline-loaded rat serum were measured as described above.

To study the effect of ROS on invasion, AH109A cells were cultured for 4 h in the absence or presence of 10 μM niacin or trigonelline and/or 4 μg/ml hypoxanthine (HX, Sigma) with 7 U/ml xanthine oxidase (XO, Sigma). To investigate the effect of trigonelline-loaded rat serum, the same treatment as described above was performed with medium containing 10% trigonelline (100 μmol/ml/100 g body weight)-loaded rat serum or vehicle (0.3% CMC)-loaded rat serum instead of the medium containing 10% CS. The treated AH109A cells were then washed once with 10% CS/MEM and seeded on the M-cell monolayer in 10% CS/MEM without niacin, trigonelline, or trigonelline-loaded serum and ROS. After culturing for 24 or 48 h, invaded cells and colonies underneath M-cells were counted with a phase-contrast microscope, as described above.

Intracellular peroxide levels in AH109A cells were assessed by flow cytometric analysis using a fluorometric probe (2',7'-dichlorofluorescin diacetate, DCFH-DA, Molecular Probes, Eugene, OR) with Epics Elite EPS (Beckman-Coulter, Hialeah, FL), as described previously.

Data were expressed as means ± SEM. Multiple comparison was performed by one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparisons test; P < 0.05 was considered statistically significant. All procedures for animal experiments in this report were conducted in accordance with guidelines established by the Animal Care and Use Committee of Tokyo Noko University, and approved by the committee.

First we examined the effect of niacin on the proliferation and invasion of AH109A cells (Fig. 1). Nicotinic acid and nicotinamide exerted no influence on the proliferation of AH109A cells at concentrations up to 40 μM in the medium. In contrast, nicotinic acid and nicotinamide commenced to suppress invasion at 2.5 μM, suppressed it approximately linearly to 20 μM, and maintained the inhibitory effect up to 40 μM. The effect of nicotinic acid was almost identical to that of nicotinamide. While trigonelline suppressed AH109A invasion at concentrations of 2.5–40 μM, it showed little influence on hepatoma proliferation at the same concentrations (Fig. 2A). As shown in Fig. 2B, rats were given oral administration of trigonelline, blood was withdrawn 0, 0.5, 1, 2, 3, 6, and 12 h later, and the effect of trigonelline-loaded rat serum on the proliferation and invasion of AH109A cells was investigated. Serum obtained 2 h after oral administration most strongly and significantly inhibited the invasion of AH109A cells, and significant inhibition was also observed at 3 and 6 h later. These results suggest that trigonelline can be absorbed from the gastrointestinal tract and that trigonelline itself or its metabolite(s) can retain their effectiveness in the blood, although the possibility that orally administered trigonelline induced an effective factor(s) originating in the host cannot be excluded. The trigonelline-loaded rat serum, however, had little effect on the proliferation of AH109A cells at any time point investigated. To examine whether niacin would inhibit the ROS-potentiated invasion of hepatoma cells, the invasion assay was performed with AH109A cells pre-cultured in ROS-containing medium. As shown in
Fig. 3, the invasive activity of AH109A cells pre-cultured in the HX–XO system which generated ROS was significantly higher than that of AH109A cells with no treatment. Nicotinic acid and nicotinamide at 10 μM inhibited the ROS-potentiated invasive activity by pre-culturing the hepatoma cells with HX and XO. Likewise, trigonelline itself (10μM) (Fig. 4A) and trigonelline (100μmole/100 g body weight)-loaded rat serum (Fig. 4B) suppressed the ROS-potentiated invasive activity of the pre-treated cells. As shown in Fig. 5, AH109A cells treated with HX–XO for 1h contained more intracellular peroxides than did control cells.
(control vs. HX–XO) when analyzed with a flow cytometer using DCFH-DA as an indicator. Trigonelline (10 μM) and trigonelline (100 μmol/100 g body weight)-loaded rat serum did not inhibit this rise in the intracellular peroxide levels of AH109A cells (HX–XO vs. HX–XO + trigonelline in Fig. 5A, HX–XO vs. HX–XO + trigonelline-loaded rat serum in Fig. 5B). Neither nicotinic acid nor nicotinamide scavenged the ROS-induced rise in the intracellular peroxide levels at 10 μM (data not shown).

In the present study, we found for the first time that niacin and trigonelline can inhibit the invasive activity
of cancer cells at low concentrations at which they exerted no influence on proliferation. Niacin, trigonelline, and trigonelline-loaded rat serum were found to inhibit ROS-induced elevation of the invasive activity of AH109A cells. In a separate experiment, we analyzed the XO activity by measuring uric acid generated by HX–XO reaction, and nicotinic acid, nicotinamide, and trigonelline were found not to affect XO activity at 10 μM, suggesting that they were not involved in the inhibition of the ROS-potentiated invasion through interference with ROS generation under the experimental conditions adopted here.

Nicotinic acid and nicotinamide are reported to have moderate radical-scavenging activities at a high concentration of 10 μM.10) Hence we measured intracellular peroxide levels after treating AH109A cells with nicacin in the absence or presence of HX–XO. But nicacin failed to suppress the ROS-induced rise in intracellular peroxide levels. The failure of nicacin to scavenge the intracellular peroxides in the present study might be due to a low concentration of 10 μM. Trigonelline was also found not to scavenge the intracellular peroxides, this being consistent with the previous finding that trigonelline had almost no scavenging ability against OH radical.10) These results suggest that the free-radical scavenging activity might not be involved in the suppressive effect of niacin and trigonelline on the ROS-potentiated invasion of hepatoma cells, unlike resveratrol,17) a polyphenol present in grapes. Coffee reportedly suppresses hepatoma cell invasion by reducing oxidative stress in vitro.9) Thus it is unlikely that trigonelline is responsible for the inhibitory action of coffee on hepatoma invasion by way of scavenging ROS. Other components such as chlorogenic acid and caffeic acid18) might be responsible for the suppressive effect of coffee on invasion through reduction of oxidative stress.

The niacin concentration in normal human plasma is minute: reported to be under 0.4 μM.19) If this be true of CS or RS, then the final concentration of niacin derived from the serum in the control medium that contains 10% CS or RS is estimated to be less than 0.04 μM. This concentration range is below 1/250 of 10 μM, which is the least effective dose of niacin, suggesting that niacin derived from serum might be negligible from the pharmacological point of view. In contrast, the basal medium, MEM, provides nicotinamide to the medium at a final concentration of about 8 μM. This concentration is comparable to the least effective dose of nicotinamide (10 μM). We found that nicotinamide did not inhibit XO activity up to 100 μM. Thus nicotinamide derived from both serum and MEM in the control medium (approximately 8 μM) might not exert any influence on XO activity, and probably not on invasive activity. As to the latter activity, we need to confirm this using nicotinamide-free MEM.

Potentiation of invasive activity of AH109A cells by ROS is reportedly mediated by the autocrine/paracrine loop of hepatocyte growth factor (HGF),16) which is known as a cell motility factor.20) Hence there is a possibility that niacin and trigonelline suppress the ROS-induced increase in AH109A invasion by interrupting this loop through, for instance, an inhibition of HGF receptor phosphorylation. Since prostaglandins are known to enhance the invasion of cancer cells,21,22) another possibility that niacin and trigonelline interrupt prostaglandin synthesis cannot be ruled out. Further studies including those on other possibilities are needed to clarify the exact modes of action of niacin and trigonelline on hepatoma cell invasion.

In conclusion, we found for the first time that niacin, trigonelline, and trigonelline-loaded rat serum inhibited the invasion of AH109A cells without affecting the proliferation of the cells, and suppressed the ROS-potentiated invasive capacity of hepatoma cells. The mechanisms by which niacin and trigonelline inhibit invasion remain to be elucidated.

References


