Note

Apoptosis induced by Picolinic Acid-related Compounds in HL-60 Cells

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We have found that niacin-related compounds, particularly picolinic acid, induced apoptosis in human leukemia cells. In this paper, we investigated whether various picolinic acid-related compounds had apoptosis-inducing activities or not. Particularly, fusaric acid, picolinaldehyde, nicotinaldehyde, 2-aminopyridine, and 3-aminopyridine also induced apoptosis in HL-60 cells. These results suggest that pyridine substituted with various groups and the consequent change of resonance structure may have an important role in the induction of apoptosis.

Key words: niacin; picolinic acid; NAD; apoptosis; HL-60

Niacin, which is known as nicotinic acid and nicotinamide in general, is a water-soluble vitamin and is converted to NAD in vivo. NAD is an important coenzyme in oxidation-reduction reactions to obtain energy, and is also a substrate for ADP-ribosylation enzymes. Poly(ADP-ribose)lation, which is catalyzed by poly(ADP-ribose) polymerase (EC 2.4.2.30: PARP), is a post-translational modification for nuclear proteins in many eukaryotic cells and has been thought to be associated with many important cellular processes, particularly DNA repair and apoptosis.1,2) It is known that caspase-3 (like) protease cleaves one of its substrates, PARP, to generate 89-kDa and 25-kDa fragments during apoptosis in many eukaryotic cells.3) Apoptosis is gene-directed cell death with distinct morphological and biochemical features in comparison with necrosis, and is induced by such stimuli as hormones, growth factor withdrawal, oxidative stress, DNA damaging reagents, and antitumor drugs which kill tumors through the induction of apoptosis.4)

We have been investigating various physiological and pharmacological functions of niacin and its related compounds in various organisms. Recently, we found that niacin-related compounds, particularly picolinic acid, dipicolinic acid, and isonicotinamide, induced apoptosis in HL-60 cells5,6) and K562 cells7) via the caspase pathway. However, in normal human quiescent lymphocytes, apoptosis was not induced by these compounds under the same conditions.7) Picolinic acid is synthesized from tryptophan in a side pathway of NAD biosynthesis in animals, and exists in various organisms as a natural component. Moreover, it is reported that picolinic acid has a growth-stimulating effect in rats8) and may improve the immune system.9) It is interesting that picolinic acid, which has various positive effects on normal cells and individuals, induces apoptosis in tumor cells. In this study, we investigated whether various picolinic acid-related compounds had apoptosis-inducing activities to obtain information about the structural features in picolinic acid as inducers of apoptosis.

HL-60 cells were cultured in RPMI 1640 medium (Gibco BRL Co., Grand Island, NY, U.S.A.) with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 10% heat-inactivated (56°C, 30 min) fetal bovine serum (Gibco BRL Co., lot US177246) at 37°C in a humidified atmosphere of 5% CO2 in air. To induce apoptosis, HL-60 cells were cultured under same conditions as described above and were treated with 0.01–10 mM various picolinic acid-related compounds for the indicated time. Picolinic acid, fusaric acid, and picolinic acid N-oxide were dissolved in PBS (−). 2-Picoline, 3-picoline, 2-pyrindinemethanol, picolinaldehyde, nicotinaldehyde, 2-aminopyridine, and 3-aminopyridine were dissolved in DMSO. Benzoic acid, DL-piperidine carboxylic acid, pyrazineamide, and pyrazine carboxylic acid were dissolved in ethanol. The solvent was used at a concentration not greater than 0.5% in all experiments. In these conditions, we confirmed that apoptosis was not induced by each solvent.

Apoptosis assays were done by the protocol described in ref. 5. Apoptosis was measured by the percentage of cells with hypodiploid DNA using a FACSCalibur (Becton Dickinson Co., San Jose, CA, U.S.A.). To detect the apoptotic DNA ladder, HL-60 cells were suspended in lysis buffer after treatment with picolinic acid-related compounds, then centrifuged. The resulting supernatant was treated with

Abbreviations: PARP, poly(ADP-ribose) polymerase; FCM, flow cytometry; DMSO, dimethyl sulfoxide

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RNase A (Sigma Chemical Co., St. Louis, MO, U.S.A.), then with proteinase K. A sample was electrophoresed in 2% agarose gel. Morphological changes of nuclei were observed under a fluorescence microscope. The treated cells were fixed with 1% glutaraldehyde and stained with 1 mM Hoechst 33342 (Calbiochem Co. Cambridge, MA, U.S.A.). Other reagents used in this research were purchased from Nacalai Tesque (Kyoto, Japan), Tokyo Kasei Kogyo (Tokyo, Japan), and Wako Pure Chemical Industries Co. (Osaka, Japan) unless otherwise specified.

At first, HL-60 cells were treated with various picolinic acid-related compounds at 5 mM for 24 h. In this condition, the percentage of apoptosis induced by picolinic acid was higher than 90%. Particularly, fusaric acid, picolinaldehyde, nicotinaldehyde, 2-amino pyridine, and 3-amino pyridine had a strong function of DNA fragmentation in HL-60 cells as analyzed by FCM as shown in Fig. 1. It is interesting that not only picolinaldehyde but also nicotinaldehyde induced apoptosis because nicotinic acid did not in our previous investigation. Moreover, we investigated the abilities of these compounds to induce apoptosis at different concentrations (Fig. 1). The percentage of DNA fragmentation caused by fusaric acid, picolinaldehyde, and nicotinaldehyde was higher than 50% at 0.5 mM. In the case of 2-amino pyridine and 3-amino pyridine, the percentage of DNA fragmentation was about 50% at 1 mM. Furthermore, picolinic acid did not induce apoptosis under these conditions. Apoptotic DNA ladders and chromatin condensation were observed in HL-60 cells after treatment with these compounds (data not shown). Therefore, these compounds induced apoptosis similarly to the case of picolinic acid.

In this study, we found that picolinaldehyde (No. 2 in Fig. 2), nicotinaldehyde (No. 3 in Fig. 2), 2-amino pyridine (No. 4 in Fig. 2), and 3-amino pyridine (No. 5 in Fig. 2) induced apoptosis. In the case of not only the carboxyl group but also aldehyde group and amino group substituted pyridine ring, apoptosis may be induced more effectively. Interestingly, in case of a highly reactive group substituted pyridine ring at C3 position as nicotinaldehyde and 3-amino pyridine, apoptosis was also induced more effectively though nicotinic acid did not. Moreover, picoline (Nos. 7 and 8 in Fig. 2) and 2-pyridinemethanol (No. 11 in Fig. 2) did not induce apoptosis. Consequently, the highly reactive group, a substituted pyridine ring may have a principal role as apoptosis inducers (Fig. 2).

The fact that picolinic acid N-oxide (No. 9 in Fig. 2) did not induce apoptosis was very interesting. Moreover, benzoic acid (No. 10 in Fig. 2), pyrazine carboxylic acid (No. 12 in Fig. 2), and DL-piperidine carboxylic acid (No. 14 in Fig. 2) had no apoptosis-inducing activities in HL-60 cells, suggesting that not only the pyridine ring may be necessary to induction of apoptosis by picolinic acid (No. 1 in Fig. 2), but also the resonant structure of the pyridine ring may be important (Fig. 2).

Interestingly, fusaric acid (No. 6 in Fig. 2), i.e. 5-n-butyl-2-picolinic acid, induced apoptosis effectively at a low concentration. This compound is known as an antibiotic isolated from Fusarium heterosporum by Yabuta et al. in 1934. In our investigation, niacin-related compounds generally induced apoptosis at a comparatively high concentration. Even picolinic acid, dipicolinic acid, and isonicotinamide did not induce apoptosis at 1 mM. We consider that this result...
may be related to the low permeability of niacin-related compounds through the cell membrane due to the structure and electrical charge of these compounds. Therefore, the introduction of a fat-soluble chain to pyridine ring may be more effective to develop drugs inducing apoptosis at a low concentration.

Our results suggest that fusaric acid, picolinaldehyde, nicotinaldehyde, 2-aminopyridine, and 3-aminopyridine induce apoptosis in HL-60, may be the key to explain the mechanism of apoptosis induced by picolinic acid. Of course, it may be necessary to consider that the apoptosis may be induced by these compounds via a different mechanism than that with picolinic acid. We need to explain the mechanism of apoptosis induced by picolinic acid further. We expect that these results will be a step in the development of antitumor therapeutics based on picolinic acid, which is a natural component in our body, with no harmful side effects, and our results might be useful in various fields in the near future.

References