Apoptosis Induced by Niacin-Related Compounds in K562 Cells but not in Normal Human Lymphocytes

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In our previous study, we found that niacin-related compounds induced apoptosis in human acute myelomonocytic leukemia cells, HL-60. We have investigated whether these compounds acted as inducers of apoptosis also in various other cell types. In human chronic myelogenous leukemia cells, K562, which are relatively resistant to various inducers of apoptosis, the apoptosis was induced by picolinic acid and dipicolinic acid in about 50% of the cells 5–10 mm via the caspase pathway, but was not at 1 mm. However, isonicotinamide did not induce apoptosis effectively in K562 cells. On the other hand, in normal human quiescent lymphocytes, the apoptosis was not induced by these compounds at the same concentrations. It is suggested that these compounds may induce apoptosis mainly in tumor cells. The change of intracellular peroxide levels was observed in the early phase of apoptosis induced by niacin-related compounds. We expect to make use of niacin-related compounds in the field of medicine.

Key words: apoptosis; niacin; K562 cells; normal human lymphocytes; antitumor drug

Apoptosis is a gene-directed active process with distinct biochemical and morphological features such as fragmentation of DNA and formation of apoptotic bodies etc. These biological events with apoptosis are induced by stimuli such as hormones, growth factor withdrawal, oxidative stress, DNA-damaging reagents, and antitumor drugs, which have been thought to kill tumors through the induction of apoptosis.1) It is well-known that the cascade of caspases (cysteine aspartate-specific proteases) plays a critical role during various kinds of apoptosis.2)

Nicotinic acid and nicotinamide, i.e. niacin, are one of water-soluble vitamins and are converted to NAD in vivo. NAD is an important coenzyme in oxidation-reduction reactions, and is also a substrate for poly(ADP-ribose) polymerase (EC 2.4.2.30: PARP). Poly(ADP-ribose)ylation is a post-translational modification for nuclear proteins and has been thought to be associated with many important cellular processes, particularly apoptosis and DNA repair.3–6)

Moreover, niacin itself has many physiological and pharmacological functions in various organisms.7–9) We have suggested that niacin and its related compounds might have various unknown functions because these compounds existed in many organisms as natural components. It is important to investigate new functions of niacin-related compounds for their future use in the fields of medicine, nutrition, etc. In our previous study, we investigated the effects of niacin on DNA repair in normal human quiescent lymphocytes. As the results, nicotinic acid promoted DNA repair synthesis, but nicotinamide inhibited it.9) We also investigated whether niacin-related compounds acted as inducers of apoptosis. Particularly, we found that picolinic acid, dipicolinic acid, and isonicotinamide induced apoptosis in HL-60 cells, but niacin did not.10) It is interesting that niacin and its related compounds, which are natural components mainly found in the NAD cycle, promote DNA repair in normal cells and induce apoptosis in human leukemia cells. In this study, we investigated whether picolinic acid, dipicolinic acid, and isonicotinamide acted as inducers of apoptosis in other human leukemia cells, K562, and normal human lymphocytes.

Materials and Methods

Cell culture and treatments. Human chronic myelomonocytic leukemia cells, HL-60, were cultured in a liquid mixture of FCS and M199, 10% human serum, and a suspension of polybrene (10 μg/mL) in a complete culture medium (Gibco). Human quiescent lymphocytes, normal human lymphocytes, K562 cells, and human acute myelogenous leukemia cells, HL-60, were cultured in RPMI-1640 media (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco) and a suspension of polybrene (10 μg/mL). A suspension of polybrene was added to each culture medium and was kept overnight at 37°C to allow the cells to absorb the polybrene. Subsequently, the cells were washed twice with phosphate-buffered saline (PBS) and then treated with each compound to induce apoptosis. Z-Asp-CH2-DCB, benzylloxycarbonyl-Asp-CH2-OOC-2, 6-dichlorobenzene; PA, picolinic acid; dipA, dipicolinic acid; isoNAm, isonicotinamide; AcD, actinomycin D; DCFH-DA, 2', 7'-dichlorofluorescin diacetate; ROS, reactive oxygen species

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Abbreviations: PARP, Poly(ADP-ribose) polymerase; FCM, flow cytometry; Z-Asp-CH2-DCB, benzylloxycarbonyl-Asp-CH2-OOC-2, 6-dichlorobenzene; PA, picolinic acid; dipA, dipicolinic acid; isoNAm, isonicotinamide; AcD, actinomycin D; DCFH-DA, 2', 7'-dichlorofluorescin diacetate; ROS, reactive oxygen species
elogenous leukemia cells, K562, and human myeloid leukemia cells, HL-60, were cultured in RPMI 1640 medium (Gibco BRL Co., Grand Island, NY) supplemented 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 38N9543) at 37°C in a humidified atmosphere of 5% CO2 in air. Normal human lymphocytes were isolated from heparinized peripheral blood from healthy human volunteers by density gradient centrifugation with Ficoll-Paque (Pharmacia Biotech. Co., Uppsala, Sweden), and were suspended in RPMI 1640 medium as described above. The cells were treated with 1–10 mM niacin-related compounds for the indicated time. As a positive control, K562 cells were treated with 1 μg/ml actinomycin D for the indicated time. To examine the effects of caspase inhibitor on apoptosis, cells were treated with 100 μM Z-Asp-CH2-DCB (Peptide Institute, Inc., Osaka, Japan) for 1 h before the treatment with niacin-related compounds.

Apoptosis assays. Apoptosis assays were performed by the protocol described in Ref. 10. To detect DNA ladder with agarose gel electrophoresis, treated cells were suspended in lysis solution (10 mM Tris-Cl, 10 mM EDTA, 0.5% Triton X-100, pH 7.5) for 10 min on ice, then centrifuged. The supernatant was treated with RNase A (Sigma Chemical Co., St. Louis, MO) and then with proteinase K. Electrophoresis was performed in 2% agarose gel.

Quantification of apoptosis was done by calculating the percentage of cells with hypodiploid DNA using flow cytometry (FCM). Cells were gently suspended in hypotonic solution [50 μg/ml propidium iodide (Sigma Chemical Co.), 3.4 mM sodium citrate, 1 mM Tris-Cl, 0.1 mM EDTA, 0.1% Triton X-100]. The percentage of apoptosis was analyzed using FACS Calibur (Becton Dickinson Co., San Jose, CA).

Detection of intracellular peroxide levels by flow cytometry (FCM). Intracellular peroxide levels were measured using the protocol of Hockenbery et al.11 Cells (5 × 10^6 cells/ml) were incubated with 5 μM 2’, 7’-dichlorofluorescin diacetate (DCFH-DA; Molecular Probes, Eugene, OR) for 1 h at 37°C before treatment with niacin-related compounds. Samples were analyzed using FACS Calibur (Becton Dickinson Co., San Jose, CA). Debris was excluded from analysis by the conventional scatter-gating method. Each analysis was performed on at least 10000 events. The experiments were repeated several times, yielding essentially identical results. Data analyses were performed using the Cell Quest software (Becton Dickinson Co.).

Other reagents used in this research were purchased from Nacalai Tesque Inc. (Kyoto, Japan), Wako Pure Chemical Industries Co. (Osaka, Japan), or Tokyo Chemical Industries Co. (Tokyo, Japan) unless otherwise specified.

Results and Discussion

At first, we investigated whether picolinic acid, dipicolinic acid, and isonicotinamide also induced apoptosis in K562 cells. Figure 1 shows that an apoptotic DNA ladder was observed in K562 cells only when treated with picolinic acid and dipicolinic acid at 10 mM for 12 and 24 h. We also observed chromatin condensation in K562 cells when treated with picolinic acid and dipicolinic acid (data not shown). Interestingly, DNA ladder and chromatin condensation were not clearly observed in K562 cells when treated with isonicotinamide.

We investigated whether these compounds induced apoptosis in a dose-dependent manner. As shown in Fig. 2, picolinic acid and dipicolinic acid induced apoptosis in K562 cells at the concentration of 5 and 10 mM, but not at 1 mM, and the percentage of apoptosis was finally about 40–50% at 24 h. A wide spectrum caspase inhibitor, Z-Asp-CH2-DCB, completely blocked the apoptosis induced by picolinic acid and dipicolinic acid.

From these results, we suggested that these compounds might induce apoptosis mainly in tumor cells. Therefore, we investigated whether picolinic acid, dipicolinic acid, and isonicotinamide induced apoptosis in normal human quiescent lymphocytes. Barbieri et al. reported that d-ribose and 2-deoxy-d-ribose induced apoptosis in normal human quiescent lymphocytes and various cell types, though quiescent lymphocytes are relatively insensitive to apoptosis.12

![Fig. 1. Agarose Gel Electrophoresis of DNA Extracted from K562 Cells after Treatment with Picolinic Acid, Dipicolinic Acid, and Isonicotinamide.](image)
Picolinic acid, dipicolinic acid, and isonicotinamide did not induce apoptosis in normal human quiescent lymphocytes although d-ribose and 2-deoxy-d-ribose induced apoptosis (Fig. 2). Moreover, d-ribose and 2-deoxy-d-ribose induced apoptosis in HL-60 and K562 cells in our investigation (data not shown). Consequently, our results suggest that these compounds may induce apoptosis in tumor cells, but not in normal cells.

It is well-known that K562 cells are relatively resistant to various inducers of apoptosis although apoptosis can be induced easily by various stimuli under the same conditions in HL-60 cells. Interestingly, the apoptosis-inducing activity of picolinic acid and dipicolinic acid might be almost equal to the activity of actinomycin D as a positive control in K562 cells under the same conditions (Fig. 2). Moreover, isonicotinamide did not effectively induce apoptosis in K562 cells although isonicotinamide induced apoptosis in HL-60 cells. We have paid attention to this result because we considered that these facts might be related to the difference of response to isonicotinamide in apoptosis between K562 and HL-60 cells and be key points in explaining the death
mechanism of apoptosis induced by picolinic acid, dipicolinic acid, and isonicotinamide. Chronic myelogenous leukemia (CML), e.g., K562 cells, which express p210 Bcr-Abl, are highly resistant to antileukemic-drug-induced apoptosis. Moreover, Amarante-Mendes et al. reported that HL-60/Bcr-Abl cells, which were stably transfected with the bcr-abl gene, were resistant to various inducers of apoptosis. Wagner et al. reported that K562 cells were resistant to membrane damage and oxidative stress. We also found that hydrogen peroxide did not induce apoptosis in K562 cells as opposed to HL-60 cells (unpublished data). Therefore, we consider that the apoptosis induced by isonicotinamide may be related to the difference of response to oxidative stress. We investigated the change of intracellular peroxide levels in the early phase of apoptosis induced by niacin-related compounds. As shown in Fig. 3, the continuous increase of intracellular peroxide levels was observed when treated with picolinic acid and dipicolinic acid in HL-60 and K562 cells. On the other hand, a transient increase was observed when treated with isonicotinamide in HL-60 cells, but not in K562 cells up to 12 h after treatment (data not shown). Moreover, no change of intracellular peroxide levels was observed when treated with picolinic acid and isonicotinamide in normal human lymphocytes. Interestingly, the continuous increase of intracellular peroxide levels was observed when treated with dipicolinic acid, which did not induce apoptosis in normal human lymphocytes.

 Reactive oxygen species (ROS) are known to induce oxidative stress and apoptosis in various cell types. Moreover, it has been suggested that ROS might play an important role as a common mediator of apoptosis induced by various stimuli. From these results, we consider that yielding ROS in the early phase of apoptosis induced by niacin-related compounds may be the various types. Particularly, ROS generated by isonicotinamide may induce membrane damage. We observed a difference of apoptosis induced by isonicotinamide between HL-60 cells and K562 cells. Moreover, yielding ROS when treated with dipicolinic acid may not be related to the process of apoptosis. We need to explain the mechanism of apoptosis induced by niacin-related compounds and particularly to investigate whether ROS generated by niacin-related compounds play an important role during apoptosis. At first, it is interesting that we examine the effect of various anti-oxidants on the apoptosis by niacin-related compounds.

Moreover, the function of NAD as the coenzyme of the oxidation-reduction reactions in mitochondria is very important. Marini et al. reported that DNA strand breakage could induce the critical consumption of NAD and ATP due to poly (ADP-ribose)lation and resulted in the decrease of GSH content. The depletion of GSH is thought to induce oxidative stress and to result in the apoptosis. Therefore, intracellular NAD level may be a key factor in the regulation of the redox status. We also need to investigate the relationship between intracellular NAD level and mitochondrial changes in the apoptosis induced by niacin-related compounds.

In summary, we suggested that niacin-related compounds induced apoptosis especially in tumor cells, but not in normal cells. Particularly, 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 rats and may improve the immune system. It is interesting that picolinic acid, which has various positive effects on normal cells and individuals, induce apoptosis in tumor cells. We want to make use of niacin-related compounds as harmless antitumor drugs. We expect that our investigations will be the first step to develop antitumor therapeutics with no harmful side effects.

References

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