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研究ノート

Comparison of antiproliferative effects of trichothecene mycotoxins, nivalenol and deoxynivalenol, in cultured cells

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Abstract

To elucidate the mechanisms underlying the toxicities of nivalenol (NIV) and deoxynivalenol (DON), their potencies on cell proliferation in cultured cells were investigated. Assays were performed after 24 h of treatment. Both toxins retarded proliferation of all 4 cell lines tested. NIV was more potent than DON in the human promyelocytic leukemia cell line HL60, the human lymphoblastic leukemia cell line MOLT-4, and the rat aortic myoblast cell line A-10. In contrast, both toxins exhibited almost the same potencies in the human hepatoblastoma cell line HepG2. If both toxins exert their toxicities through the same mechanism, ratios of the 50 % inhibitory concentration (IC_{50}) of DON to NIV are expected to be constant regardless of the types of cells used in the assays. However, the ratios of each IC_{50} varied, indicating differences in the mechanism of action of these toxins.

Key words: cell proliferation; deoxynivalenol; nivalenol

Introduction

A variety of *Fusarium* fungi produce a number of trichothecenes, a significant class of mycotoxins. At present, more than 100 trichothecene mycotoxins are known; nivalenol (NIV) and deoxynivalenol (DON) are 2 such trichothecenes, which have similar chemical structures. The *Fusarium* fungi are commonly found on cereals grown in temperate regions (Creppy, 2002). In Japan, NIV contamination as well as DON contamination of cereals is commonly found (Nakajima and Yoshida, 2007); hence, studies on both NIV and DON toxicities are very meaningful. Leucopenia is one of the leading signs of trichothecene toxicosis (Joffe, 1971), implying that trichothecenes hinder cell proliferation. Indeed, we have demonstrated NIV-caused retardation of cell proliferation in human leukemia HL60 cells (Nagashima et al., 2006). Besides, cell proliferation is one of the most fundamental biological phenomena. In this study, therefore, to elucidate the mechanism underlying the toxicities of NIV and DON, we focused on their effects on cell proliferation in various cultured cells and compared the potencies of both toxins.

Materials and Methods

Chemicals and cells

NIV and DON were purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in dimethyl sulfoxide. A

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colorimetric immunoassay kit (Cell Proliferation ELISA, BrdU [colorimetric]) was purchased from Roche Diagnostics GmbH (Penzberg, Germany). The human promyelocytic leukemia cell line HL60 and the human hepatoblastoma cell line HepG2 were purchased from RIKEN Cell Bank (Tsukuba, Japan). The human acute lymphoblastic leukemia cell line MOLT-4 was purchased from Health Science Research Resources Bank (Sennan, Japan). The rat aortic myoblast cell line A-10 was purchased from American Type Culture Collection (Manassas, VA). HL60 and MOLT-4 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen Corp, Carlsbad, CA) containing 10 % fetal calf serum (FCS; JRH Biosciences Inc, Lenexa, KS). A-10 and HepG2 cells were cultured in Dulbecco's Modified Eagle's Medium (Nissui Pharmaceutical Co, Ltd, Tokyo, Japan) containing 10 % FCS (JRH Biosciences).

Cell proliferation

Cell proliferation was investigated by measuring 5-bromo-2-deoxyuridine (BrdU) incorporation during DNA synthesis, as described previously (Nagashima and Goto, 2000). Approximately 1.5×10^4 cells (HL60) or 2×10^3 cells (MOLT-4, A-10, and HepG2) in 100 μL of medium containing either NIV or DON were placed in each well of a 96-well microtiter plate, and cell proliferation was assessed after 24 h of culture.

Results and Discussion

DON hindered cell proliferation in HL60 cells and the 50 % inhibitory concentration (IC_{50}) was 0.36 ± 0.09 μg/mL (1.22 μM) in 5 replicates. Considering that the IC_{50} of NIV was 0.16 μg/mL (0.51 μM) in HL60 cells (Nagashima et al., 2006), DON is less potent than NIV concerning the cell proliferation in this cell line. Though the IC_{50} was slightly lower, MOLT-4 cells exhibited similar results to HL60 cells (Fig. 1). Because both cell lines are categorized as leukemia cell lines, this coincidence would be accounted for by the cell type. To our knowledge, this is the first report describing the effects of trichothecene mycotoxins on MOLT-4 cells. The results of the assays with A-10 cells showed the same trend as in the former 2 cell lines; that is, NIV is more potent than DON (Fig. 1). However, the IC_{50} of DON in A-10 cells

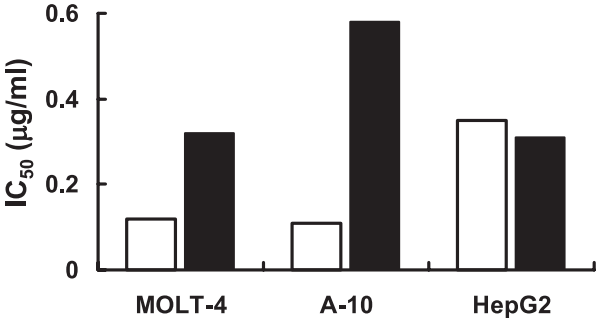

Fig. 1 Antiproliferative effects of trichothecene mycotoxins on MOLT-4, A-10, and HepG2 cells

Cells were treated with either nivalenol (NIV) or deoxynivalenol (DON) for 24 h. Open and filled bars represent treatment with NIV and DON, respectively. Each cell proliferation assay was examined in triplicate. IC_{50} stands for 50 % inhibitory concentration.

was higher than that in the former 2 cell lines; consequently, the ratio of the IC_{50} of DON to that of NIV in A-10 cells was higher than that in the former 2 cell lines. To our knowledge, there is no report on the effects of trichothecene mycotoxins on A-10 cells, although a different type of the trichothecene mycotoxin T-2 toxin was reported to retard cell proliferation in primary cultured cells derived from aortic smooth muscles (Yaron et al., 1987). The IC_{50} of DON in HepG2 cells was similar to that in HL60 and MOLT-4 cells, while the IC_{50} of NIV was evidently higher than that in other cell lines (Fig. 1). Contrary to the results from other cell lines, the potency of DON was the same as or even higher than that of NIV in HepG2 cells. With regard to cell proliferation, NIV is more potent than DON in most of the cells tested (Johannisson et al., 1999; Luongo et al., 2008; Minervini et al., 2004; Severino et al., 2006; Taranu et al., 2010; Thuvander et al., 1999), indicating that HepG2 is an exception. Sahu et al. (2010) investigated the effects of DON on the viability of HepG2 cells; however, they did not address the effect on cell proliferation.

Supposing that both NIV and DON exert their toxicities through the same mechanism and that the only difference between NIV and DON is the potency of their toxicity, the ratios of the IC_{50} of DON to that of NIV are expected to be almost the same, regardless of the cell line. However, the ratio of the IC_{50} ranges from 0.9 (HepG2) to 5.3 (A-10), indicating that there are some differences in

the mechanisms underlying the toxicities of NIV and DON.

In the present study, we investigated the effects of NIV and DON on cell proliferation in various cultured cells and demonstrated the differences of toxicities between these toxins. However, further studies are required to identify what factor(s) contributes to the difference.

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培養細胞におけるトリコテセン系マイコトキシンのニバレノールとデオキシニバレノールの細胞増殖阻害活性の比較

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要　約

ニバレノール（NIV）とデオキシニバレノール（DON）の毒性発現機構を解明するために，培養細胞の増殖に対する両毒素の阻害効果を検討した．毒素処理24時間後に試験を行った．両毒素は試験に供した４つの培養細胞の増殖を遅らせた．ヒト前骨髄球白血病細胞 HL 60とヒトリンパ芽球白血病細胞 MOLT-4，ラット大動脈筋芽細胞 A-10においては，DON よりも NIV の方が効果が強かった．これに対し，ヒト肝芽腫細胞 HepG 2では両毒素ともほぼ同じ効果を示した．もし両毒素が同一の毒性発現機構で毒性を発揮しているのであれば，細胞の種類にかかわらず DON と NIV の50％阻害濃度（IC_{50}）の比は一定になると考えられるが，各々の細胞の IC_{50}の比は一様でないことから，両毒素の毒性発現機構には違いがあると考えられた．