Anti-Cancer Drugs 1998, 9, pp. 739–741

Short report

ND-2001 suppresses lung metastasis of human renal cancer cells in athymic mice

Yasuhiro Kuramitsu, Jun-ichi Hamada,[1] Tsutomu Tsuruoka,[2] Kiyoshi Morikawa, Seiji Naito,[3] Hiroshi Kobayashi and Masuo Hosokawa

Laboratories of Pathology and [1]Cell biology, Cancer Institute, Hokkaido University School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060-0015, Japan. [2]Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd, 760 Morooka-cho, Kohoku-ku, Yokohama 222-0002, Japan. [3]Department of Urology, Faculty of Medicine, Kyushu University, Maidashi 3 Chome, 1-1, Fukuoka 812-0054, Japan.

Explants of highly metastatic human renal cell carcinoma SN12Cpm6 cells in athymic mice were treated with sodium D-glucaro-δ-lactam (sodium 5-amino-5-deoxy-D-glucosaccharic acid-δ-lactam; ND-2001). ND-2001 (50 μg/ml) caused 78% inhibition of lung metastasis of SN12Cpm6 cells (two of five animals remaining metastasis free). The *in vitro* tumor cell invasion assay showed that ND-2001 (100 μg/ml) suppressed the invasive activity of SN12Cpm6 cells to Matrigel matrix at an inhibition rate of 72%. These results suggest that ND-2001 may be a new anti-metastatic drug against human cancer cells. [© 1998 Lippincott Williams & Wilkins.]

Key words: Invasion, metastasis, renal cell carcinoma.

Introduction

Sodium D-glucaro-δ-lactam (sodium 5-amino-5-deoxy-D-glucosaccharic acid-δ-lactam; ND-2001) is a drug that has been synthesized from the antibiotic nojirimycin.[1,2] Reports have indicated that lung metastases and invasive activities of rat and murine experimental tumors were inhibited by *in vivo* or *ex vivo* treatment with ND-2001.[3–5] Our recent study also showed that *ex vivo* treatment of rat hepatoma cKDH-8/11 cells with ND-2001 inhibited their lung metastasis significantly. We demonstrated that ND-2001 suppressed the invasive activities and haptotaxis toward laminin of cKDH-8/11 cells.[6] In the present study, we examined the inhibitory effects of ND-2001 on lung metastases and invasive activity of human renal cell carcinoma SN12Cpm6[7] with high metastatic potential in athymic mice.

This work was supported by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture, and the Ministry of Health and Welfare, Japan.

Correspondence to Y Kuramitsu, First Department of Biochemistry, Yamaguchi University School of Medicine, Kogushi 1144, Ube 755-8505, Japan. Tel: (+81) 836 22 2213; Fax: (+81) 836 22 2212

Materials and methods

Animals

Female BALB/c *nu/nu* mice, 8 weeks old, were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). The animals were kept in a barrier facility and maintained under laminar air-flow conditions with controlled temperature, humidity and 12 h light/dark cycle in the Experimental Animal Institute of the Hokkaido University School of Medicine. Food and water were supplied *ad libitum*.

Cell lines

SN12C is a human renal cell carcinoma cell line which was established in culture from a surgical specimen. SN12Cpm6 is a sub-clone obtained by *in vivo* selection of SN12C cells with high metastatic potential in nude mice.[7] The SN12Cpm6 cells were maintained in a continuous *in vitro* culture in RPMI 1640 medium (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS).

Reagent

ND-2001 was synthesized from C-1 and C-6 of the antibiotic nojirimycin via two oxidation steps.[1–3] The ND-2001 was kindly provided by Meiji Seika Kaisha (Yokohama, Japan).

Treatment of SN12Cpm6 cells with ND-2001 ex vivo

SN12Cpm6 cells (2×10^6) were seeded onto 100 mm culture dishes in 9 ml of RPMI 1640 medium supplemented with 10% FBS. An aliquot of 1 ml of ND-2001 (1 mg/ml, 500 μg/ml) was added to the cultures (final concentration of ND-2001 at 100 or 50 μg/ml). RPMI 1640 medium supplemented with 10% FBS was used as control. The cultures were incubated at 37°C in a CO_2 incubator for 24 or 48 h.

Lung metastasis of the tumor cells after treatment with ND-2001 ex vivo

SN12Cpm6 cells treated with or without ND-2001 for 24 h were harvested, washed and suspended in PBS^-. The cells (2×10^6) were implanted i.v. into the tail vein of the athymic mice. The mice were killed 77 days after tumor implantation for the examination of lung metastasis colonies. The lung was weighed before fixation in Bouin's solution and the metastasis colonies on the lung surface were counted macroscopically after fixation. The lung colonies were counted in a blind fashion by two observers.

Invasion assay

The invasive activity of the tumor cells was assayed with the use of Transwell cell culture chambers (Costar, Cambridge, MA) according to the method previously reported.[6,8-10]

Statistical analysis

Statistical determination was calculated by the Student's *t*-test.

Results

Effects of ND-2001 on lung metastasis of SN12Cpm6 cells in athymic mice

Table 1 shows that the *ex vivo* ND-2001 treatment inhibited lung metastasis of SN12Cpm6 cells in athymic mice. The number of lung metastasis colonies in the mice implanted with SN12Cpm6 cells treated with ND-2001 (50 μg/ml) *ex vivo* was significantly less than that of untreated SN12Cpm6 cells ($p < 0.01$). ND-2001 inhibited the development of lung metastasis of SN12Cpm6 cells at an inhibition rate of 78.1% (two of five animals remaining metastasis free).

Effects of ND-2001 on in vitro invasion of SN12Cpm6 cells

Table 2 shows the effects of ND-2001 on *in vitro* invasion of SN12Cpm6 cells. ND-2001 treatment (100 μg/ml) suppressed the invasion of Matrigel matrix (inhibition rate of 72.1%) by SN12Cpm6 cells.

Table 2. Effects of ND-2001 on invasion of human renal cell carcinoma SN12Cpm6 cells in vitro

Treated with ND-2001[a] (μg/ml)	Invasion assay[b]	
	No. of cells/ 0.3 mm^2	Inhibition rate (%)
0	31.2	0
100	8.7	72.1

[a]SN12Cpm6 cells were treated with ND-2001 for 48 h in vitro.
[b]SD12Cpm6 cells (3.6×10^4) were placed into the upper compartments of Transwell chambers and attracted by NIH 3T3 conditioned medium. Incubation period for invasion assay was 5 h.

Table 1. Effects of ND-2001 on the metastasis of human renal cell carcinoma SN12Cpm6 cells in BALB/c/nu/nu mice

Treatment with ND-2001[a] (μg/ml)	Lung metastasis			
	Incidence (%)	No. of colonies[b] (mean ± SD)	Inhibition rate (%)	Lung weight (g, mean ± SD)
0	4/4 (100)	>200, 172, 131, 102 (151.3 ± 37.6)	0	0.50 ± 0.28
50	3/5 (60)	112, 53, 1, 0, 0 (33.2 ± 44.4)[c]	78.1	0.24 ± 0.03
100	5/5 (100)	>200, 146, 92, 10, 8 (91.2 ± 75.3)	39.7	0.37 ± 0.19

[a]SN12Cpm6 cells (2×10^6) were treated with ND-2001 for 24 h in vitro and implanted i.v. into BALB/c/nu/nu mice.
[b]The mice were killed 77 days after tumor implantation for the examination of lung metastasis colonies.
[c]$p < 0.01$.

Discussion

The new anti-metastatic drug ND-2001 has been reported to inhibit lung metastases and invasive activity of rat and murine experimental tumors.[3-5] However, its anti-metastatic effects have not previously been examined in an athymic mouse model for human tumor. In this study, we investigated the inhibitory effect of ND-2001 on the lung metastasis of human renal cell carcinoma in the model animal. The results of this study indicate that *ex vivo* treatment with ND-2001 had significantly potent anti-metastatic effects on human renal cell carcinoma SN12Cpm6 cells. ND-2001 suppressed the invasion of Matrigel matrix by SN12Cpm6 cells at an inhibition rate of 72.1%. These results suggest that ND-2001 treatment suppressed the invasion of the basement membrane, resulting in the inhibition of lung metastases of human renal cell carcinoma cells in the same manner as previously reported by us for rat hepatoma cells.[6]

Our present study therefore suggests that ND-2001 could be a new anti-metastatic drug for human clinical application.

Acknowledgments

We wish to thank Meiji Seika Kaisha, Ltd for kindly providing ND-2001. We also thank Ms M Yanome for preparing the manuscript.

References

1. Tsuruoka T, Niwa T, Shomura T, *et al.* Synthesis of D-glucaro-δ-lactam, an oxidation product of Nojirimycin. *Sci Rep Meiji Seika Kaisha* 1973; **13**: 80–4.
2. Seftor REB, Seftor EA, Grimes WJ, *et al.* Human melanoma cell invasion is inhibited *in vitro* by swainsonine an deoxymannojirimycin with a concomitant decrease in collagenase IV expression. *Melanoma Res* 1991; **1**: 43–54.
3. Tsuruoka T, Fukuyasu H, Azetaka M, *et al.* Inhibition of pulmonary metastases in experimental tumors by D-glucaro-δ-lactam sodium salt (ND-2001). *Abstr 2nd Joint Meet AACR/JCA* 1992; No.B-3.
4. Tsuruoka T, Fukuyasu H, Azetaka M, *et al.* Inhibition of pulmonary metastasis and tumor cell invasion in experimental tumors by sodium D-glucaro-δ-lactam (ND-2001). *Jpn J Cancer Res* 1995; **86**: 41–7.
5. Tsuruoka T, Azetaka M, Iizuka Y, *et al.* Inhibition of tumor cell haptotaxis by sodium D-glucaro-δ-lactam (ND-2001). *Jpn J Cancer Res* 1995; **86**: 1080–5.
6. Kuramitsu Y, Hamada J, Tsuruoka T, Morikawa K, Kobayashi H, Hosokawa M. A new anti-metastatic drug, ND-2001, inhibits lung metastases in rat hepatoma cells by suppressing haptotaxis of tumor cells toward laminin. *Anti-Cancer Drugs* 1998; **9**: 88–92.
7. Naito S, Walker SM, Fidler IJ. *In vivo* selection of human renal cell carcinoma cells with high metastatic potential in nude mice. *Clin Exp Metast* 1989; **7**: 381–9.
8. Yamada K, Kennedy DW, Yamada SS, Gralnick H, Chen W-T, Akiyama S. Monoclonal antibody and syntetic peptide inhibitors of human tumor cell migration. *Cancer Res* 1990; **50**: 4485–96.
9. Welch DR, Lobl TJ, Seftor EA, *et al.* Use of the membrane invasion culture system (MICS) as a screen for anti-invasive agents. *Int J Cancer* 1989; **43**: 449–57.
10. Albini A, Iwamoto Y, Kleinman HK, *et al.* A rapid *in vitro* assay for quantitating the invasive potential of tumor cells. *Cancer Res* 1987; **47**: 3239–45.

(Received 14 May 1998; revised form accepted 4 June 1998)