ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Mar; 23 (3): 253-256
http://www.chinaphar.com

Effects of lidamycin on genomic DNA in human heptatoma BEL-7402 cells[1]

R96 A

HE Qi-Yang, JIANG Bing, LI Dian-Dong[2] (*Institute of Medicinal Biotechnology, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100050, China*)

KEY WORDS lidamycin; antineoplastic agents; DNA damage; *ras* genes; hepatoma

ABSTRACT

AIM: To further study the effect of enediyne antibiotic lidamycin (C1027) on genomic DNA in human hepatoma BEL-7402 cells. **METHODS**: The DNA patterns were detected by agarose gel electrophoresis. The gene damage was revealed by Southern hybridization. The DNA repair was determined by neutral agarose gel electrophoresis. The lidamycin-cleaved sites were determined with superhelix GEM plasmid. **RESULTS**: The DNA "ladder" patterns were observed after the BEL-7402 cells were treated with lidamycin 1 μmol/L for 15, 30, 60 min. The extrons and introns of active N-ras gene in the BEL-7402 cells were easily damaged by lidamycin at low concentrations, but no damage in the silent IL-2 gene was observed. It was difficult to repair DNA breaks after the BEL-7402 cells were treated with lidamycin. The cleaved sites of GEM plasmid treated with lidamycin were -OH HO-. **CONCLUSION**: Lidamycin can significantly damage genomic DNA in the hepatoma BEL-7402 cells. The results make it helpful to elucidate the molecular mechanism of potent cytotoxicities of lidamycin to tumor cells.

INTRODUCTION

Enediyne antibiotics are currently the focus of intense research due to their unique actions on DNA and potent biological activities. Lidamycin (LDM, original name C1027), a member of them, was isolated from *Streptomyces globisporus* C1027 strain in China[1]. LDM potently inhibited the growth of tumor cells *in vivo* and *in vitro*, and the replications of SV40 virus and Epstein Barr virus as well[2-5]. It has been reported that LDM can generate interstrand cross-links and cleave the DNA with sequence preference[6,7]. However, its action reveals 285 fold more efficiently in cells than in a cell-free environment[8]. So, it needs to study the details of LDM's effect on damaging genomic DNA. In this paper, we examined the effect of LDM on genomic DNA in human hepatoma BEL-7402 cells to further elucidate its mechanism.

MATERIALS AND METHODS

Cell culture and drug The human hepatoma BEL-7402 cell line was grown as a monolayer in RPMI 1640 medium (GIBCO) supplemented with 10 % calf serum, penicillin G (100 kU/L), streptomycin (100 mg/L), and glutamine (2 mmol/L) and incubated at 37 ℃ in a humidified atmosphere containing 5 % CO_2 and 95 % air. Highly purified LDM was prepared in our laboratory.

Genomic DNA isolation and agarose gel electrophoresis The genomic DNA isolation and agarose gel electrophoresis were performed as previously[9]. In brief, the cells after treatment with LDM were scraped with a cell scraper. The cell lysate was treated with RNase 100 mg/L (Sigma) and proteinase K 100 mg/L (Merck) at 50 ℃ for 3 h. The genomic DNA sample was extracted three times with phenol: chloroform. The DNA samples (15 μg/lane) were run on 1.5 % agarose gel and detected under ultraviolet illumination.

DNA isolation and Southern blot analysis High molecular weight DNA was extracted from lidamycin-treated or untreated control cells. DNA samples (3 μg/lane) were digested with *Eco*R Ⅰ for N-*ras* probe and with *Xba* Ⅰ for IL-2 probe. Digested DNA samples were electrophoresized at 80 V for 4 h on

[1] Project supported by the National Natural Science Foundation of China (No 30070878) and Major State Basic Research Development Program Foundation of China (No G2000057010).
[2] Correspondence to Prof LI Dian-Dong. Phn 86-10-6316-5289. Fax 86-10-6301-7302. E-mail ddli@public3.bta.net.cn
Received 2001-07-03 Accepted 2001-12-24

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Mar; 23 (3)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

1.0 % agarose gel, transferred to nitrocellulose filters and then hybridized with [α-^{32}P] ATP-labelled N-*ras* cDNA or IL-2 cDNA. Autoradiographes were obtained by exposure to X-film with an enhancer screen at −70 ℃ for 1 or 2 d. The N-ras genomic 8.8 kb probe was kindly provided by Dr XIA Li, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

Analysis of DNA double breaks by neutral gel electrophoresis The cells were treated with LDM for 30 min, and then divided into two groups. One group was immediately for extraction of DNA, the other was incubated for another 30 min before extraction of DNA. The high molecular DNA samples were extracted from untreated and LDM-treated cells and their DNA double breaks were detected by neutral gel electrophoresis[10]. The DNA samples (3 μg/lane) were run on 0.6 % agarose gel for 4−5 h. The gel was stained with ethidium bromide 0.5 mg/L and detected under ultraviolet illumination.

The cleaved sites of LDM on superhelix GEM plasmid The GEM plasmid (a gift of Dr John YH CHAN at MM Anderson Cancer Center in USA) was treated with LDM at 37 ℃ for 2 h and then divided into three groups: T4 polynucleotide kinase (GIBCO), T4 DNA ligase Ⅰ (GIBCO) and combination of T4 polynucleotide kinase with T4 DNA ligase Ⅰ. The reactions were undertaken for 30 min after addtion of [γ-^{32}P] ATP and T4 polynucleotide kinase and/or T4 DNA ligase Ⅰ. The reacted products were run on 1.0 % agarose gel. The gel was dried under vacuum and exposure to X-film at −70 ℃.

RESULTS

Direct cleavage of genomic DNA by LDM into a ladder pattern in whole BEL-7402 cells The DNA ladder patterns were observed after the BEL-7402 cells were treated with LDM 1 μmol/L (Fig 1). The cleavage action of LDM was detected in short time (15 min). The DNA ladders were shown obviously with the increase of treating time up to 30, 60 min.

Damaging effect on extrons and introns in N-*ras* oncogene The damaging effect of LDM on introns in N-*ras* oncogene was observed with a 8.8-kb cDNA probe (Fig 2-A). Intron damage in N-*ras* oncogene was taken place at a higher concentration of 0.1 μmol/L. The damaging effect of LDM on extrons in N-*ras* oncogene was determined with a 1.0 kb N-*ras* cDNA probe. Both 7.0 kb and 8.8 kb bands were visualized in untreated control cells; notably, a band with the length between 7.0 kb and 8.8 kb was found in the cells treated with LDM 0.01 μmol/L and 1 nmol/L for 4 h and a small band with the length about 4.0 kb was found in the cells treated with LDM 0.1 μmol/L (Fig 2-

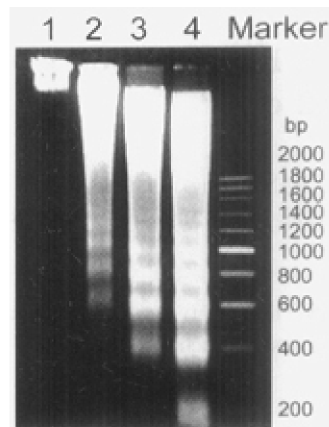

Fig 1. The DNA ladder patterns of human hepatoma BEL-7402 cells caused by direct cleavage of genomic DNA with lidamycin. The cells were treated without (Lane 1) or with lidamycin 1 μmol/L for 15 (Lane 2), 30 (Lane 3), 60 (Lane 4) min. The DNA samples were run on 1.5 % agarose gel and detected by staining with *ethidium bromide*.

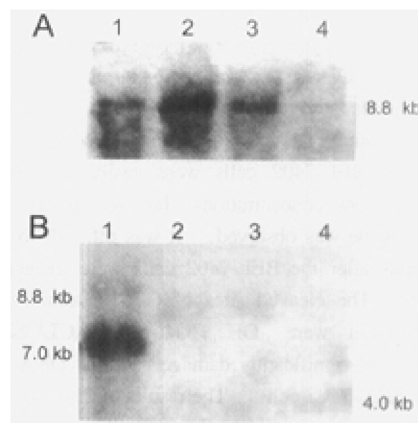

Fig 2. Damaging effect of lidamycin on introns (A) and extrons (B) of N-*ras* oncogene in human hepatoma BEL-7402 cells. In Fig 2-A, the cells were treated without (Lane 1) or with lidamycin 0.001 (Lane 2), 0.01 (Lane 3), 0.1 (Lane 4) μmol/L for 4 h. In Fig 2-B, the cells were treated without (1) or with lidamycin 0.00001 (Lane 2), 0.001 (Lane 3), 0.1 (Lane 4) μmol/L for 4 h. The probes used were a 1.0 kb fragment for extrons and 8.8 kb fragment for introns.

B). The results indicated that LDM could cause extron damage at low concentrations.

Effect on silent IL-2 gene The damaging effect of LDM on the silent IL-2 gene was determined by using a 0.6 kb IL-2 cDNA probe. LDM did not damage the silent IL-2 gene in the range of 0.001 to 0.1 μmol/L (Fig 3).

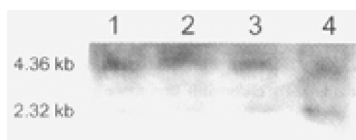

Fig 3. Damaging effect of lidamycin on IL-2 gene in human hepatoma BEL-7402 cells. The cells were treated without (Lane 1) or with lidamycin 0.001 (Lane 2), 0.01 (Lane 3), 0.1 (Lane 4) μmol/L for 4 h. The probe used was a 0.6 kb cDNA fragment of human IL-2 gene.

DNA repair of genomic DNA damaged with LDM The DNA damage was maintained evidently for 30 min after the BEL-402 cells were treated with LDM 0.1, 1, 5 μmol/L for 30 min (Fig 4). This meant that there was no DNA repair in the LDM-treated cells.

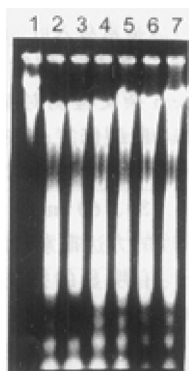

Fig 4. The DNA break repair of BEL-7402 cells detected by neural electrophoresis. The cells were treated without (1) or with lidamycin 5 (2, 3), 1 (4, 5), 0.1 (6, 7) μmol/L for 30 min. The cells in lanes 3, 5 and 7 were incubated for another 30 min.

The LDM-cleaved sites in the GEM plasmid The GEM plasmids were treated with LDM 0.03, 0.3 μmol/L for 30 min and the results were different after addition of T4 kinase and T4 ligase (Fig 5). In the T4 kinase group, the bands of GEM plasmid were observed. On the other hand, no band was shown in T4 ligase Ⅰ group. However, the intensities of the bands were reduced in combination of T4 kinase with T4 ligase Ⅰ.

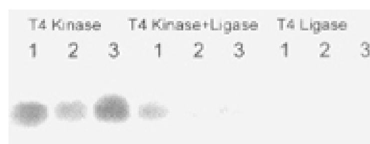

Fig 5. Damage sites caused by lidamycin were determined by T4 polynucleotide kinase and ligase Ⅰ in the GEM plasmid. 1, control; 2, lidamycin 0.03 μmol/L for 2 h; 3, lidamycin 0.3 μmol/L for 2 h.

DISCUSSION

The results showed that LDM potently damaged the genomic DNA and it was very difficult to repair the LDM-caused DNA damage. The data made it helpful to elucidate the molecular mechanism of potent cytotoxicities of LDM to tumor cells. It is the first evidence for enediyne antibiotics that LDM can directly cleave the genomic DNA into a ladder pattern in whole tumor cells. The results were in agreement with the data obtained with isolated neclei of BEL-7402 cells[11]. According to the feature of DNA cleavage, the cytotoxicity of LDM to tumor cells was stronger than that of enediyne antibiotic neocarzinostatin, the same type of nine-member enediyne. There was no DNA ladder in the human SK-N-SH neuroblastoma cells treated with neocarzinostatin (0.0016 – 10 μmol/L) for 1 h[12]. It was reported that the damages in HeLa nuclei were directly caused by a lot of enediynes with little contribution from endogenous factors, such as nucleases and topoisomerases[13].

The DNA damage produced by LDM was easily detected in transcriptionally active N-ras gene than in silent IL-2 gene. This phenomenon was observed in transcriptonally active human p53 gene and phosphoglycerate kinase gene damaged by enediyne antibiotics, esperamicin A1 and C. The DNA damage in the latter was enhanced in sequences located between several transcription factor binding sites[14]. The DNA break ends produced by LDM were -OH HO- in the GEM plasmid. The conclusion was based on the following analysis. In the T4 kinase group, the bands of GEM plasmid were observed for the T4 kinase linked the cleave site of 5′ end -OH in the plasmid. The cleaved site of 3′ end was -OH, for T4 ligase Ⅰ only joined the 5′ end with phosphorus group in the plasmid. However, the

intensities of the bands were reduced in combination of T4 kinase with T4 ligase Ⅰ, for T4 ligase Ⅰ competitively occupied the sites of 5′ end in the plasmid. The result might give an explanation for no detectable DNA repair in the hepatoma BEL-7402 cells. In conclusion, our study indicated that LDM could potently damage genomic DNA in human hepatoma BEL-7402 cells, resulting in potent cytotoxicities to tumor cells.

REFERENCES

1 Hu JL, Xue YC, Xie MY, Zhang R, Otani T, Minami Y, *et al*. A new macromolecular antitumor antibiotic, C-1027 Ⅰ. Discovery, taxonomy of producing organism, fermentation and biological activity. J Antibiot (Tokyo) 1988; 41: 1575-9.
2 Zhen YS, Ming XY, Yu B, Otani T, Saito H, Yamada Y. A new macromoleculer antitumor antibiotic, C-1027 Ⅲ. Antitumor activity. J Antibiot (Tokyo) 1989; 42: 1294-8.
3 Zhen HY, Xue YC, Zhen YS. Inhibition of angiogenesis by antitumor antibiotic C1027 and its effect on tumor metastasis. Natl Med J China 1997; 77: 657-60.
4 McHugh MM, Beerman TA, Burhans WC. DNA-damaging enediyne C-1027 inhibits initiation of intracellular SV40 DNA replication in trans. Biochemistry 1997; 36: 1003-9.
5 McHugh MM, Beerman TA. C-1027-induced alterations in Epstein-Barr viral DNA replication in latently infected cultured human Raji cells: relationship to DNA damage. Biochemistry 1999; 38: 6962-70.
6 Xu YJ, Zhen YS, Goldberg IH. C1027 chromophore, a potent new enediyne antitumor antibiotic, induces sequence-specific double-strand DNA cleavage. Biochemistry 1994; 33: 5947-54.
7 Xu YJ, Xi Z, Zhen YS, Goldberg IH. Mechanism of formation of novel covalent drug. DNA interstrand cross-links and monoadducts by enediyne antitumor antibiotics. Biochemistry 1997; 36: 14975-84.
8 Cobuzzi RJ, Kotsopoulos SK, Otani T, Beerman TA. Effects of the enediyne C-1027 on intracellular DNA targets. Biochemistry 1995; 34: 583-92.
9 He QY, Zhang HQ, Pang DB, Chi XS, Xue SB. Resistance to apoptosis of harringtonine-resistant HL60 cells induced by tetrandrine. Acta Pharmacol Sin 1996; 17: 545-9.
10 Bianchi NO, Bianchi MS, Lopez-Larraza D, Alitalo K, de la Chapelle A. Damage and repair induced by bleomycin in the domain of human amplified MYC oncogenes. Cancer Res 1990; 50: 2379-84.
11 Xu YJ, Li DD, Zhen YS. The molecular mechanism of new antitumor antibiotic C1027 having potent cytotoxicity toward tumor cells. Sci Sin (B) 1992; 22: 814-9.
12 Yu L, Goldberg IH, Dedon PC. Enediyne-mediated DNA damage in nuclei is modulated at the level of the nucleosome. J Biol Chem 1994; 269: 4144-51.
13 Hartsell TL, Yalowich JC, Ritke MK, Martinez AJ, Schor NF. Induction of apoptosis in murine and human neuroblastoma cell lines by the enediyne natural product neocarzinostatin. J Pharmacol Exp Ther 1995; 275: 479-85.
14 Wu J, Xu J, Dedon PC. Modulation of enediyne-induced DNA damage by chromatin structures in transcriptionally active genes. Biochemistry 1999; 38: 15641-6.

力达霉素对人肝癌 BEL-7402 细胞基因组 DNA 的影响[1]

何琪杨，姜 兵，李电东[2]
（中国医学科学院中国协和医科大学医药生物技术研究所，北京 100050，中国）

关键词 lidamycin；抗肿瘤药；DNA 损伤；*ras* 基因；肝细胞瘤

目的：深入研究力达霉素对人肝癌 BEL-7402 细胞基因组 DNA 的影响．**方法**：琼脂糖凝胶电泳检测 DNA 泳带；Southern 杂交检测对基因的损伤；中性凝胶电泳检测 DNA 断裂修复；超螺旋 GEM 质粒检测断裂 DNA 位点．**结果**：力达霉素 1 μmol/L 处理 BEL-7402 细胞 15、30、60 min，可检测到明显的 DNA 梯带．低浓度的力达霉素对活跃基因 N-*ras* 的外显子和内含子均有明显的损伤作用，但对沉默 IL-2基因没有影响．力达霉素断裂 DNA 后，DNA 损伤难以修复．力达霉素断裂 GEM 质粒 DNA 的位点是-OH HO-．**结论**：力达霉素明显地损伤人肝癌 BEL-7402 细胞的基因组 DNA．该结果有助于解释其高活性地杀伤肿瘤细胞的分子机制．

（责任编辑 吴民淑）