ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 May; 20 (5): 391 – 394
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

6,7-Dimethoxycoumarin attenuated cisplatin-induced DNA interstrand crosslink and DNA-protein crosslink in primary cultured rabbit kidney proximal tubular cells

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KEY WORDS 6,7-dimethoxycoumarins; cisplatin; rabbits; proximal kidney tubules; cultured cells; DNA; proteins; crosslink

ABSTRACT

AIM: To study the mechanism of cisplatin interaction with DNA, and the attenuating effects of 6,7-dimethoxycoumarin (DMOC) on crosslink. **METHODS**: Primary cultured rabbit kidney proximal tubular cells (PTC) were established. DNA interstrand crosslink was assayed with ethidium bromide binding and DNA-protein crosslink with ^{125}I-postlabelling. PTC were incubated with cisplatin for 24 h. DMOC was preincubated with PTC for 24 h, and cisplatin (26 μmol·L^{-1}) was added into culture and incubated for another 24 h. **RESULTS**: Cisplatin induced formation of DNA interstrand crosslink (13, 26, 52, and 78 μmol·L^{-1}) and DNA-protein crosslink (26, 52, and 78 μmol·L^{-1}) ($P<0.01$). DNA interstrand crosslink in DMOC (0.4, 4, and 8 mg·L^{-1}) and DNA-protein crosslink in DMOC (4, 8 mg·L^{-1}) were less than those in cisplatin group (26 μmol·L^{-1}), respectively ($P < 0.01$). **CONCLUSION**: The mechanisms of cisplatin interaction with DNA in PTC were DNA interstrand crosslink and DNA-protein crosslink, and DMOC attenuated these effects *in vitro*.

INTRODUCTION

Cisplatin was an effective antitumor agent, but its serious nephrotoxicity limited its clinical use. Cisplatin suppressed duplication of DNA[1], expression of mRNA[2], and synthesis of protein[3] in kidney cells. However, carboplatin did not suppress duplication of DNA, and injure cell nucleus, in addition, nephrotoxicity of carboplatin was less than that of cisplatin[4]. Thus, DNA was the target responsible for cisplatin nephrotoxicity. Mechanism of kidney cell nucleus injury might relate to DNA interstrand crosslink and DNA-protein crosslink because cisplatin-induced DNA interstrand crosslink and DNA-protein crosslink caused tumor cell nucleus injury[5]. 6,7-Dimethoxycoumarin (DMOC) could relieve the toxicity of mechlorethamine[6] and cyclophosphamide[7] which induced DNA interstrand crosslink. But the mechanism was unknown. Perhaps DMOC could also relieve nephrotoxicity of cisplatin, which induced DNA interstrand crosslink and DNA-protein crosslink like mechlorethamine[8] and cyclophosphamide[9].

Therefore, in the present study, the mechanism of cisplatin interaction with DNA, and the attenuating effects of 6,7-dimethoxycoumarin (DMOC) on crosslink were investigated in primary cultured rabbit kidney proximal tubular cells (PTC).

MATERIALS AND METHODS

RPMI-1640 culture medium was obtained from Gibco (USA). HEPES and ethidium bromide were purchased from Flank (Swiss). β-Mercaptoethanol was obtained from Sigma (USA). ^{125}I was made in Chinese Atomic Energy Isotope Co. New Zealand white rabbits (about 1 month old, ♂ or ♀) were obtained from the Center of Laboratory Animal (Third Military Medical University). Cisplatin was obtained from Shandong Qilu Pharmaceutical Factory. DMOC (purity 90.5 %) was obtained from Mr WAN Yao-De, Sichuan Institute of Chinese Materia Medica.

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Received 1998-07-13 Accepted 1998-10-29

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 May; 20 (5)
E-mail aps@server shcnc.ac.cn Phn Fax 86-21-6474-2629

Primary cultures of kidney PTC Primary cultures of rabbit kidney PTC were prepared by an established procedure[10]. In brief, the renal cortices were dissected free, finely minced, sequentially ground and filtered through 216 μm, 172 μm, and 108 μm non-rust steel mesh. The proximal tubules on 172 μm and 108 μm non-rust steel mesh were harvested, and incubated with RPMI-1640 (10 % fetal bovine serum) at 37 ℃ in 5 % CO_2.

Identified PTC Alkaline phosphatase of brush border in PTC was stained by cytochemistry according to Gomori Ca-Co, and epithelium keratin was identified by anti-keratin[11].

Experimental groups (1) Control group, without cisplatin and DMOC; (2) Cisplatin group, incubation of PTC with cisplatin 13, 26, 52, and 78 μmol·L^{-1} for 24 h; (3) DMOC + cisplatin group, after preincubation of PTC with DMOC (0.4, 4, and 8 mg·L^{-1}) for 24 h, cisplatin (26 μmol·L^{-1}) was added into culture and incubated for another 24 h.

Ethidium bromide binding assay of DNA interstrand crosslink The method was established accroding to Huang JM[12]. In brief, the DNA of cells on 24-mutiwell plate was dissolved in the resuspension buffer (Tris 10 mmol·L^{-1}, edetic acid 0.1 mmol·L^{-1}, SDS 0.5 %, RNase 100 μg). The buffer of two wells was merged (total volume 500 μL), 250 μL of the buffer was transfered into a tube which contained 3 mL ethidium bromide solution (ethidium bromide 0.5 mg·L^{-1}, edetic acid 2 mmol·L^{-1}, Na_2HPO_4 20 mmol·L^{-1}, pH 12). The fluorescence (F_1) of the reaction solution was measured using a fluorometer at 532 nm excitation (slit width 10 nm) and 586 nm emission (slit width 10 nm). Then, the reaction solution was heated in a boiling water bath incubator at 100 ℃ for 10 min, immediately cooled to room temperature and fluorescence (F_2) was measured. The DNA interstrand crosslink (ISC) index was calculated as follows:

$$ISC = (-\ln X_{treated}) - (-\ln X_{control})$$

Where, $X = (F_1 - F_2)/F_1$

^{125}I-postlabelling assay of DNA-protein crosslink DNA-protein crosslink (DPC) was measured by ^{125}I-postlabelling[13]. In brief, residual polytides and amino acid associated with DNA (5 – 10 μg) were postlabelled by ^{125}I, precipitated, and unincorporated ^{125}I in the supernatant was washed off. The DNA samples were assayed for radioactivity in a γ-counter and the same samples were used for measurement of UV absorbance at 260 nm/280 nm. If the ratio of the absorbance at 260 nm to 280 nm were higher than 1.75, the samples would not be polluted by protein. DPC was expressed as Bq/g DNA.

Statistical analysis Results were expressed as $\bar{x} \pm s$. Newman-Keuls test was used.

RESULTS

Cells were stained black color by cytochemistry according to Gomori Ca-Co (Fig 1A) and epithelium keratin was stained brown by anti-keratin (Fig 1B). These results showed that cultured cells were PTC.

ISC was increased from 0.02 in 0 μmol·L^{-1} of cisplatin to 0.87 in 13 μmol·L^{-1} of cisplatin ($P < 0.01$), and then with concentration of cisplatin increased, ISC was increased continually. When concentration of cisplatin attained to 78 μmol·L^{-1}, ISC was increased to 3.52 ($P < 0.01$). There was no significant difference of Bq/g DNA between 0 μmol·L^{-1} and 13 μmol·L^{-1} ($P > 0.05$). When concentration of cisplatin attained to 26 μmol·L^{-1}, Bq/g DNA in 13 μmol·L^{-1} of cisplatin was increased to 10 times that in 0 μmol·L^{-1} ($P < 0.01$). However, Bq/g DNA in 78 μmol·L^{-1} of cisplatin was 20 times that in 0 μmol·L^{-1} ($P < 0.01$) (Tab 1).

Preincubation of PTC with DMOC for 24 h, ISC

Tab 1. Cisplatin-induced DNA interstrand crosslink (ISC) and DNA-protein crosslink (Bq/g DNA) in PTC, and DMOC influenced these. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* cisplatin 0 μmol·L^{-1} group. $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* cisplatin 26 μmol·L^{-1} group.

	ISC ($n = 4$)	GBq/g DNA ($n = 5$)
Cisplatin/μmol·L^{-1}		
0	0.02 ± 0.04	1.4 ± 0.6
13	0.87 ± 0.07^c	3.8 ± 0.7^a
26	1.53 ± 0.09^c	20.0 ± 2.3^c
52	2.95 ± 0.3^c	22.5 ± 3.4^c
78	3.52 ± 0.3^c	38.4 ± 6.3^c
DMOC + cisplatin (26 μmol·L^{-1})		
0.4 mg·L^{-1}	1.25 ± 0.05^{cf}	22.0 ± 3.0^d
4 mg·L^{-1}	0.57 ± 0.12^{cf}	14.2 ± 3.2^{cf}
8 mg·L^{-1}	0.22 ± 0.07^{cf}	15.1 ± 3.0^{cf}

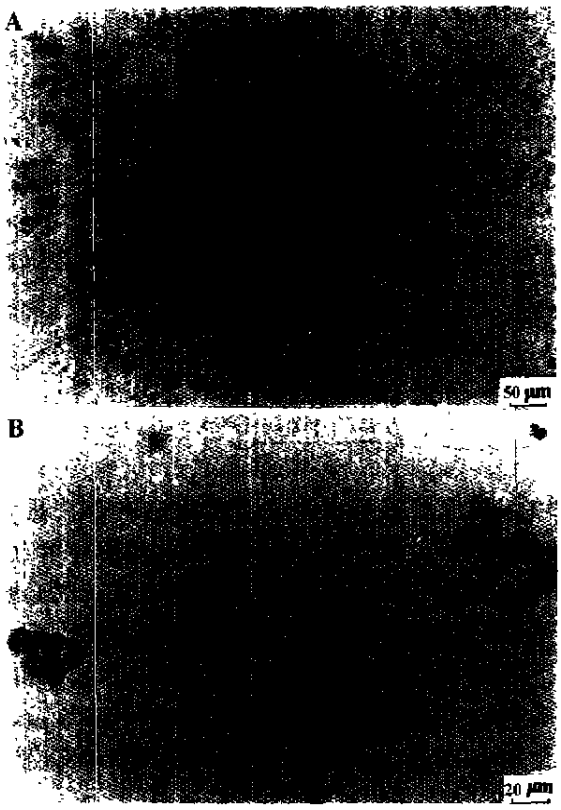

Fig 1. A) Cells were stained black color (× 40). B) Epithelium keratin was stained brown by anti-keratin (× 100).

in DMOC (0.4 mg·L^{-1}) + cisplatin (26 μmol·L^{-1}) group was decreased to 81 % of that in cisplatin group (26 μmol·L^{-1}) ($P < 0.01$), but there was no difference of Bq/g DNA between DMOC (0.4 mg·L^{-1}) + cisplatin (26 μmol·L^{-1}) group and cisplatin (26 μmol·L^{-1}) group ($P > 0.05$); ISC and Bq/g DNA in DMOC (4 mg·L^{-1}) + cisplatin (26 μmol·L^{-1}) group, were decreased to 45 % and 71 % of those in cisplatin (26 μmol·L^{-1}) group, respectively ($P < 0.01$). ISC and Bq/g DNA in DMOC (8 mg·L^{-1}) + cisplatin (26 μmol·L^{-1}) group, were decreased to 14 % and 75 % of those in cisplatin (26 μmol·L^{-1}) group ($P < 0.01$), respectively (Tab 1).

DISCUSSION

It was found that DNA interstrand crosslink and DNA-protein crosslink were induced in PTC by cisplatin. These results showed that the ways of interaction between DNA of kindey cells and cisplatin were DNA interstrand crosslink and DNA-protein crosslink. Cisplatin-induced DNA interstrand crosslink and DNA-protein crosslink could suppress duplication of DNA, expression of mRNA, and synthesis of protein in kidney cells, and even kill kidney cells. Thus, cisplatin-induced DNA interstrand crosslink and DNA-protein crosslink were responsible for cisplatin nephrotoxicity.

DMOC reduced cisplatin-induced DNA interstrand crosslink and DNA-protein crosslink in PTC, thus, DMOC was an antidote of DNA crosslinking agents and DNA-protein crosslinking agents. These effects might be related to the mechanism of protecting against cisplatin-induced nephrotoxicity and other DNA crosslinking agent-induced toxic effects such as mechlorethamine and cyclophosphamide. DNA interstrand crosslink and DNA-protein crosslink were important genotoxic lesions induced by environmental agents and carcinogens such as UV light, γ-radiaton, alkylating agents, formaldehyde, nickel, and chroamte[5,13,14]. Thus, DMOC could be used as an antidote to attenuate toxicity of these environmental agents and carcinogens to protect genetic material. In conclusion, the ways of cisplatin interaction with DNA in PTC were DNA interstrand crosslink and DNA-protein crosslink, and DMOC attenuated these as an antidote of DNA interstrand crosslink and DNA-protein crosslink.

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ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 May; 20 (5)
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茵陈素减弱顺铂导致原代兔肾小管上皮细胞 DNA 链间交联和 DNA-蛋白交联

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关键词 6,7-二甲氧基香豆素；顺铂；兔；肾近端小管；培养的细胞；DNA；蛋白质；交联

目的：研究顺铂与肾近端小管 DNA 的作用机制，和茵陈素的干预作用. **方法**：原代培养兔肾近端小管细胞(PTC)，溴乙锭荧光测 DNA 链间交联，^{125}I 标记测 DNA-蛋白交联. 顺铂与 PTC 保温 24 h，茵陈素与 PTC 提前 24 h 保温后，加入顺铂 26 μmol·L^{-1}再保温 24 h. **结果**：顺铂 13 到 78 μmol·L^{-1}和 26 到 78 μmol·L^{-1}可使 PTC 形成 DNA 链间交联和 DNA-蛋白交联. 茵陈素(0.4，4，8 mg·L^{-1})和(4，8 mg·L^{-1})组，DNA 链间交联和 DNA-蛋白交联分别低于顺铂(26 μmol·L^{-1}). **结论**：顺铂导致肾近端小管形成 DNA 链间交联和 DNA-蛋白交联，茵陈素减弱这两种作用.

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