ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Nov; 20 (11): 975-978
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Comet electrophoresis of blood nucleated cells in genotoxicity assessment

GUO Ju-Yuan, TU Zeng-Hong[1] (*State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS agar gel electrophoresis; *L*-4-oxalysine; 10-hydroxycamptothecin; norpregnenes; dimethyl sulfoxide; bleomycin; mitomycin C; lymphocytes; cell survival

ABSTRACT

AIM: Genotoxicity evaluations of several different chemicals including *L*-4-oxalysine, 10-Hydroxycamptothecin (HCT), 19-norprogesteron (ST1435), dimethyl sulfoxide (Me_2SO), bleomycin (BLM), and mitomycin C (MMC). **METHODS**: Alkaline comet assay *in vitro* (single cell gel) (SCG). **RESULTS**: *L*-4-oxalysine and HCT did not cause directly DNA damage. ST1435, the subdermal implant progestin, had no effect on DNA damage until the dose level up to 4 mmol·L^{-1}. Me_2SO did not increase DNA damage at concentration below 2 %, but showed a concentration-dependent DNA damage at $\geq$4 %. Bleomycin and mitomycin C demonstrated a strong dose-dependent DNA damage. **CONCLUSION**: Comet assay as a tool to test the genotoxicity of suspected chemicals, is rapid, simple, sensitive, good reproducible, and inexpensive.

INTRODUCTION

Owing to the exquisite sensitivity, the low cost, reproducibility, and its application in measuring DNA damage in individual cells, the comet assay becomes an important tool in predicting the genotoxicity of chemicals[1]. In this paper, Me_2SO, *L*-4-oxalysine, HCT (10-hydroxycamptothecin), ST1435 (19-norprogesteron), bleomycin, and mitomycin C were tested for their ability to induce DNA damage in rat blood cells *in vitro* using the alkaline comet assay to further evaluate the characteristics of this method.

Me_2SO is a very commonly used solvent for chemicals. As far as we know there is no published report about the genotoxicity of Me_2SO in the comet assay. We are interested in whether Me_2SO induces DNA damage and what concentration is suitable to use as a solvent in the comet assay.

L-4-Oxalysine, H_2-CH_2-CH_2-O-CH_2-CH(NH)$_2$-COOH (a white powder, purity 99 %) was first isolated from *Streptomyces roseoviridofuscus* n. sp. at our institute. This compound could inhibit the growth of several kinds of experimental tumors[2]. So it was of interest to evaluate whether *L*-4-oxalysine acted on DNA.

10-Hydroxycamptothecin (HCT) was first isolated from *Camptotheca acuminata* Decaisne in China (a yellow fluid, purity >90 %). HCT has a demonstrated anticancer effect both in animal experiments and clinical treatment[3,4]. Several reports show that HCT has strong genotoxicity[3,4]. Its anticancer function is due to inhibition of DNA topoisomerase I[5], without effects on DNA.

ST1435, a synthetic progestin, is an ineffective contraceptive when given orally[6,7,8]. As is well known, an increased incidence of cancers in pituitary and mammary glands, and neoplastic changes in the liver are most commonly observed after treatment with estrogen, progestogen, and estrogen-progestogen in many rodent species[8]. In addition, some steroidal drugs induces sister chromatid exchanges (SCE), chromosome aberrations (CA), and micronuclei (MN)[9].

In this study, we investigated the genotoxic potential of Me_2SO, *L*-4-oxalysine, HCT, ST1435

[1] Correspondence to Prof TU Zeng-Hong.
Phn 86-21-6431-1833 ext 325. Fax 86-21-6437-0269.
E-mail zhtu@server.shcnc.ac.cn
Received 1998-11-23 Accepted 1999-05-04

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Nov; 20 (11)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

using the alkaline comet assay with rat blood white cells, using bleomycin and mitomycin C as positive controls, and saline and blank as negative controls.

MATERIALS AND METHODS

Chemicals and rats ST1435, a white powder (purity 99 %), was dissolved in Me_2SO (E Merch, D-6100 Darmstadt, FR Germany) at the designed concentrations. *L*-4-oxalysine and HCT were from our institute. Bleomycin and mitomycin C were bought from Nippon Kayako Co, and Kyowa Hakko Kogyo Co, Tokyo, Japan, respectively. Bleomycin (BLM), mitomycin C (MMC), *L*-4-oxalysine, and HCT were dissolved in physiologic saline at needed concentration.

Twenty SD rats (Grade Ⅱ, № 117, 7 – 8 wk, $n = 20$, 180 – 200 g weight) obtained from the Experimental Animal Center of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Rats were housed in cages with stainless steel grid tops and floors in a room of 25 ± 2 ℃, humidity 55 – 70 %, and light on 8:00 – 20:00. Diet and tap water were supplied *ad lib* by hopper. The rats were assigned randomly to either the control or treatment groups. All rats received a 7-d acclimatization before the experiment.

Alkaline comet assay The rats were anesthetized by intraperitoneal injection of pentobarbital sodium (Serva) at 40 $mg \cdot kg^{-1}$. The abdominal aorta blood was collected in a heparinized vacutainer. The blood was then aliquot into 0.5 mL staples in 1.5 mL centrifuge tubes. Whole blood was treated for 1 h with the test substances at 37 ℃, and processed immediately in the SCG test.

SCG test was performed as described by Hartmann and Speit[10] with minor modifications. Cell lysing, DNA unwinding, and electrophoresis were performed at 4 ℃ and analysed using a fluorescence microscope (Leitz Diaplan) (excitation filters 530 – 560, suppression filter 580, dichromatic mirror 580) within 24 h. Images of 100 randomly selected cells ($n = 3$ replicate slides) were analyzed from each sample at 250-fold magnification. Cells were graded by eye into 2 categories corresponding to the amounts of DNA in the tail: no damaged (< 5 %), and damaged (5 – 95 %) according to the criteria of D Anderson[11] (Fig 1).

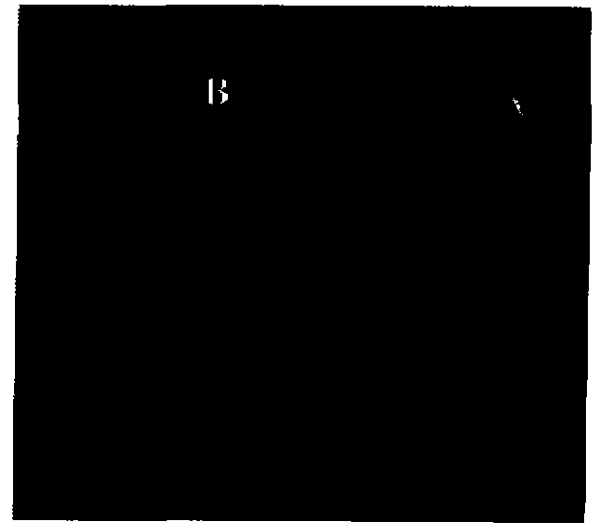

Fig 1. Fluorescence comet image of rat lymphocyte at basal level (A) or with a long tail (B).

Statistical methods Analysis was performed by a slide reader (GJY), and tested for significance ($\alpha = 0.05$, two tails) using chi-square test.

Cell viability assay After a 1-h treatment of the blood cells with test compounds the cell viability was tested using trypan blue dye (Chroma) exclusion.

RESULTS

The cell viability of each treated group was more than 90 %.

The extended DNA migration of cells exposed to physiologic saline and in the blank control were not much different ($P > 0.05$), so we combined them as a control positive DNA damage rate: 4 % ± 0.3 %, its 95 % confidence interval was 3 % – 5 %.

Me_2SO ≤2 % did not produce any DNA damage, whereas the concentrations ranging from 4 % to 40 % induced DNA damage ($P < 0.05$) in a concentration-dependent manner.

Increases in DNA damage were also seen concentration-dependently for MMC at concentrations of 2 – 6 $mmol \cdot L^{-1}$, and for BLM at concentrations of 10-57 $\mu mol \cdot L^{-1}$ (Fig 2).

No increase of DNA damage was seen in rat blood cells after treatment with *L*-4-oxalysine and HCT, even at concentrations as high as 370 $mmol \cdot L^{-1}$ and 22.8 $\mu mol \cdot L^{-1}$ respectively (Fig 3).

ST1435 was not soluble in water, and so 2 %

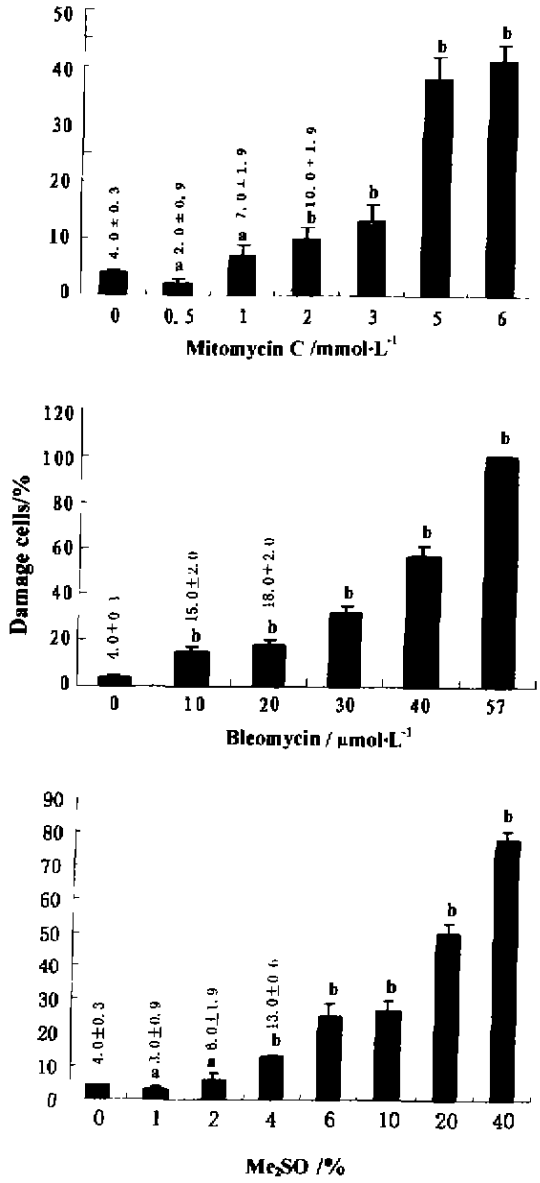

Fig 2. Rate of damage in rat blood cells (SCG test) treated with Me_2SO (solvent), MMC, and BLM (positive controls). $n = 3 \times 100$ replicate slides. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control.

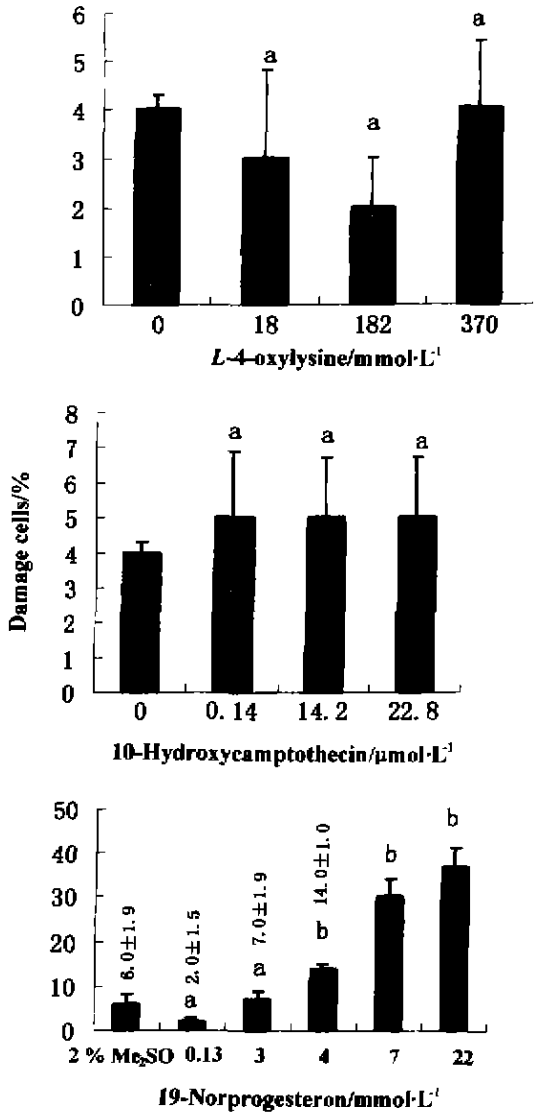

Fig 3. Rate of damage in rat blood cells (SCG test) treated with *L*-4-oxalysine, HCT, and ST1435. $n = 3 \times 100$ replicate slides. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control.

Me_2SO was used as solvent. ST1435 below 3 mmol·L^{-1} did not increase DNA damage, but it increased the damage at concentrations of 4 – 22 mmol·L^{-1} ($P < 0.05$, Fig 3).

DISCUSSION

Comet assay, as a tool to test the genotoxicity of suspected chemicals, is rapid, simple, sensitive, good reproducible, and inexpensive.

Me_2SO did not increase DNA damage until concentrations as great as 4 % were tested, So when using Me_2SO as the solvent, the concentration should be $\leqslant 2$ %.

BLM was more sensitive than MMC when using comet assay to test DNA damage. BLM induced marked cell damage at 10 μmol·L^{-1} while MMC at 2 mmol·L^{-1}.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Nov; 20 (11)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

L-4-oxalysine inhibited the growth of several different kinds of experimental tumors[2], but it did not show any genotoxic activity[12]. Our previous study showed that the IC_{50} of *L*-4-oxalysine for CHL cell was 18 mmol·L^{-1}, but only at 4 mmol·L^{-1}, the cell metaphase was observed[12]. In view of the wide distance between IC_{50} and the observed metaphase doses, no alter notion of circular dichroism DNA spectra[12] and inability in inducement of DNA damage as shown in this study, the mechanism of action is left open.

HCT, an antitumor drug, induced chromosomal aberrations in CHO cell[4] with an lg_{50} of 0.59 mg·L^{-1} (1.62 μmol·L^{-1}). At this dose, HCT inhibited cell division completely; at 0.125 mg·L^{-1} (0.34 μmol·L^{-1}), the cells appeared good metaphase[3,4], without effect on circular dichroism DNA spectra[4]. In this study, 1.4 μmol·L^{-1}(0.5 mg·L^{-1}) and 22.8 μmol·L^{-1} did not show any increase of DNA damage. HCT was supposed to induce genotoxicity only in dividing cells. Rat blood nucleated cells are normally in G_0 phase, inhibition of Topoisomerase Ⅰ is out of the question.

ST1435 did not induce significant increase in DNA damage at concentration of less than 4 mmol·L^{-1}($P < 0.05$) much higher than the serum concentration inhibiting ovulation 56-139 pmol·L^{-1}[13].

ACKNOWLEDGMENT The authors thank Dr PANG Bing and Dr CHEN Shen-Ling (School of Public Health, Shanghai Medical University) for their technical help.

REFERENCES

1 Fairbairn DW, Olive PL, O'Neill KL. The Comet assay a comprehensive review. Mutat Res 1995; 339: 37 – 59.
2 Li XY, Yong LM, Jin YF, Cao CY, Liang HZ, Wang JF. Immunopotentiating effects of oxalysine. Acta Pharmacol Sin 1987; 8: 173 – 7.
3 Tu ZH, Wang MY, Xiao WQ, Qi XD, Tang WD. Effects of 10-hydroxycamptothecin on induced chromosome aberrations in Chinese hamster ovary cells and micronuclei in mouse bone marrow and fetal liver. Acta Pharmacol Sin 1990; 11: 378 – 81.
4 Tu ZH, Wu HY, Wang MY. The comparison of mutagenicity with 10-hydroxycamtothecin in human lymphocytes and in prokaryotic cells. Carcinog Teratog Mutagen 1994; 6: 15 – 20.
5 Potinesil M. Camptothecins. From bench research to hospital wards. Cancer Res 1994; 54: 1431 – 9.
6 Laurikka-Routti M, Haukkamaa M. A contraceptive subdermal implant releasing the progestin ST1435: ovarian function, bleeding patterns, and side effects. Fertil Steril 1992; 58: 1142 – 7.
7 Heikinheimo O, Noe G, Haukkamaa M, Lahteenmaki P. The progestin ST1435-rapid metabolism in man. Contraception 1994; 50: 275 – 89.
8 Dhillon VS, Ohillon IK. Genotoxicity evaluation of norethisterone acetate. Mutat Res 1996; 367: 1 – 10.
9 Hundal BS, Dhillon VS, Sidhu IS. Genotoxic potential of estrogens. Mutat Res 1997; 389: 173 – 81.
10 Hartmann A, Spert G. Genotoxic effects of chemicals in the single cell gel (SCG) test with human blood cells in relation to the induction of sister-chromatid exchanges (SCE). Mutat Res 1995; 346: 49 – 56.
11 Anderson D, Yu T-W, Phillips BJ, Schmezer P. The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the comet assay. Mutat Res 1994; 307: 261 – 71.
12 Tu ZH, Ni HN, Wu HY, Wang MY. Influence of oxalysine on the mitosis of CHL cells and its genotoxic study. Carcinog Teratog Mutagen 1995; 7: 332 – 5.
13 Haukkamaa M, Laurikka-Routti M, Heikinjeimo O, Moo-Young A. Conception with subdermal implants releasing the progestin ST1435: A dose-finding study. Contraception 1992; 45: 49 – 55.

975-978

血细胞彗星电泳在遗传毒性评价中的应用

R965.2

郭菊媛，屠曾宏 (中国科学院上海药物研究所国家新药研究重点实验室，上海 200031，中国)

关键词 琼脂凝胶电泳；*L*-4-氧代赖氨酸；10-羟基喜树碱；烯诺孕酮；二甲基亚砜；博莱霉素；丝裂霉素 C；淋巴细胞；细胞存活

血细胞彗星电泳 遗传毒性

目的：用彗星实验检测不同种类化学物在单细胞水平上 DNA 的损伤情况. **方法**：体外彗星实验. **结果**：*L*-4 氧代赖氨酸及 10-羟基喜树碱不能直接引起 DNA 的损伤. ST1435 剂量高达 4 mmol·L^{-1}时才能对 DNA 产生损伤. 二甲基亚砜，作为一种常用溶剂，当其浓度小于 2 %时，不增加对 DNA 的损伤，但当浓度≥4 %时，可产生具有剂量-效应关系的 DNA 损伤. 博莱霉素和丝裂霉素对 DNA 有强的剂量-效应关系. **结论**：慧星实验作为检测可疑化合物遗传毒性的工具，具有快速，灵敏，简单，重复性好及价廉的优点.

(责任编辑 周向华)