Environmental mutagens & Carcinogens 16-1 : 6-12 (1996)

Chromosomal Aberration Assay of Taxol and 10-deacetyl baccatin III in Chinese Hamster Lung Cells *In Vitro*

Jae-Chun Ryu*, Kyung-Ran Kim, Eun-Kyung Ryu, Hyun-Joo Kim, Oh-Seung Kwon Choong-Eui Song, Woongchon Mar*** and Il-Moo Chang*****

*Toxicology Laboratory, Doping Control Center, **Division of Applied Chemistry, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul 130-650, Korea,*

****Natural Products Research Institute, Seoul National University, 28 Yeongun-dong, Chongno-ku, Seoul, Korea*

(Received January 26, 1996)

ABSTRACT : **To investigate the clastogenicity of taxol and its precursor, 10-deacetyl baccatin III, we performed chromosomal aberration assay with chinese hamster lung cells *in vitro*. The IC_{50} values of taxol and 10-deacetyl baccatin III were determined as $1/16\times10^{-4}$ M (5.34 μg/ml) and 1×10^{-2} M (560 μg/ml) in MTT assay, respectively. It means that the cytotoxicity of taxol revealed 100 times more cytotoxic than 10-deacetyl baccatin III in chinese hamster lung cell line. Nevertheless the strong positive genetic toxicity of taxol in the bone marrow micronucleus assay *in vivo* which was recently reported, we observed weak positive clastogenicity of taxol only in the absence of metabolic activation system in the concentration ranges used in this experiment. Moreover, to clarify the involvement of metabolic fate of taxol because of its strong positive result *in vivo*, 10-deacetyl baccatin III which is a precursor in taxol synthesis, also subjected in chromosomal aberration assay *in vitro*. However, we observed no clastogenicity of 10-deacetyl baccatin III in this experiment. From above results, it was suggested that the esterification at C-13 appears to be relative for its genetic toxicity in chromosome aberration using chinese hamster lung cell *in vitro*.**

Key Words : Taxol, 10-deacetyl baccatin III, Clastogenicity, Chromosome Aberration, Chinese Hamster Lung Cell, *In Vitro*

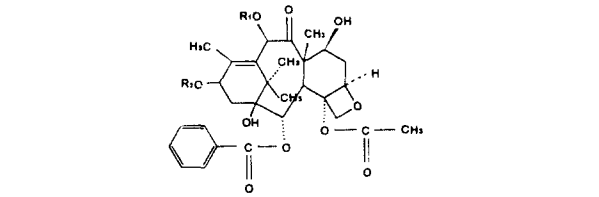
Introduction

Several assay systems having rapidity and reliability have been introduced for the evaluation of genetic hazard in humans, such as reversion test with bacterial gene mutation (Ames *et al.*, 1973, 1975; Maron and Ames, 1983), chromosomal aberration assay with mammalian cells (Ishidate and Odashima, 1977; Matsuoka *et al.*, 1979; Radman *et al.*, 1982; Jenderny *et al.*, 1988; OECD, 1993), and micronucleus assay with rodents (Hayashi *et al.*, 1982, 1990, 1992, 1994). These assay systems are now well used to evaluate the genotoxicity of chemicals and to predict the carcinogenicity *in vivo*, and also frequently adopted as methods for an index of genotoxicity in worldwide. Our laboratory have also been involved in these toxicological researches, especially in genetic toxicity (MOE report, 1992, 1993, 1994, 1995). In this respect, we reported no clastogenicity of major trichothecene mycotoxins such as T-2, HT-2 toxin, nivalenol, deoxynivalenol (Ryu *et al.*, 1993a) and some chemicals (Ryu *et al.*, 1994a, 1994b, 1996) using chinese hamster lung fibroblast cells *in vitro*.

In general, the majority of antineoplastic agents are also carcinogenic to human, so it is important to assess the carcinogenic potential of new antineoplastic agents. Recently, it was reported that the etoposide which is cancer chemotherapeutic human carcinogen was a potent inducer of micronucleus in male rat (Lahdetie *et al*, 1994).

Taxol is a constituent which was isolated from the bark of *Taxus brevifolia*. It was identified as an anti-cancer agent for

*To whom all correspondences should be addressed.



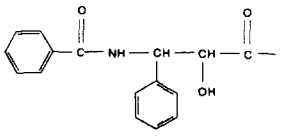
	R_1	R_2
Taxol	CH_3CO-	
10-Deacetyl baccatin III	H-	H-

Fig. 1. Chemical structures of taxol and 10-deacetyl baccatin III.

the treatment of ovarian and breast carcinomas (Wani *et al.*, 1971; Kingston *et al.*, 1990). It is a potent inhibitor of eukaryotic cell replication, blocking cells in the late G_2-mitotic phase of the cell cycle. It has been shown to stabilize microtubules in the absence of GTP, which is normally required for assembly, and presumed to prevent cell division by inhibiting the normal functions of cellular microtubule and mitotic spindles, resulting in cell death (Burkhart *et al.*, 1994; Horwitz, 1992). These activities of taxol are in contrast to the known activities of other mitotic spindle poisons such as colchicine and vinblastine, which also inhibit microtubule assembly *in vitro* (Schiff *et al*, 1979).

Baccatin derivatives are the starting material for the chemical synthesis of taxol-like anti-cancer agents. 10-Deacetyl baccatin III (DAB) is also natural compound similar with taxol in structure (Kingston *et al.*, 1990; Horwitz, 1992). It was deacetylated at C-10 position of baccatin III. The chemical structure of taxol and DAB is illustrated in Fig. 1. It was also reported that taxol was active in inhibiting cell growth, but DAB was inactive at the concentration of 20 μM in macrophage-like cell line J774.2 (Parness *et al.*, 1982). Therefore, it was suggested that the esterification at C-13 appears to be essential for its activity.

Despite of the literature review (Bissett & Kaye, 1993), research in chemistry (Kingston 1991), pharmacological effects (Rowinsky & Donehower, 1991) and phase I clinical trial (Wiernik *et al.*, 1987), the genetic toxicity of taxol and baccatin derivatives has not been reported in the literature. However, recently Tinwell and Ashby (1994) reported that taxol gave a strong positive response in the mouse bone marrow micronucleus assay as the first report of its genotoxicity. They reported that taxol induced micronucleus significantly, about 8 times higher than control at the dose of 50 mg/kg, ip, in mice.

So we aim to investigate the clastogenicity in mammalian cells and to identify the mutagenic moiety in taxol structure compared with DAB in the presence and absence of rat liver S-9 metabolic activation system in chinese hamster lung cells *in vitro*.

Materials and Methods

The methods used in this experiment was performed as described by OECD (1993) and Ishidate *et al* (1977) with some minor modifications (Ryu *et al.*, 1994b, 1996) which are briefly summarized as follows.

Cell Culture

A clonal sub-line of a chinese hamster lung (CHL) fibroblast cells was obtained from the National Institute of Health Sciences, Tokyo, Japan. The karyotype of CHL cells consisted of 25 chromosomes. The cells had been maintained by 3-4 day passages and grown in a monolayer with Eagle's minimum essential medium (EMEM, Gibco, 410-1100EA) supplemented with 10% fetal bovine serum (FBS, Gibco, 26140-020). These cells were maintained at 37°C in 5% CO_2 atmosphere.

Reagents

Trypsin-EDTA and colcemid were the products of Gibco BRL Life Tech. Inc. (Gaithersburg, USA). The test chemicals, taxol and 10-deacetyl baccatin III, were purchased from the Sigma Chemical Company and Janssen Chimica, respectively. The test compounds were dissolved in dimethylsulfoxide (DMSO). The preparation of rat liver S-9 fraction for metabolic activation system was reported previously (Ames *et al.*, 1973; Maron and Ames, 1983). The S-9 fraction prepared stored immediately at -80°C before use.

Determination of the 50% growth inhibition concentration

Test article dose levels were determined prior to the main

study in a dose range-finding study performed both in the presence and absence of a rat liver S-9 activation system. For the growth inhibition assay, CHL cells were seeded at the densities of 1×10^4 cells/0.2 ml into 96 well plates. Twenty-four hours after seeding, several different doses of sample were separately added and incubated for 24 hours. And then the 50% inhibition concentration (IC_{50}) values were calculated by MTT assay (Mosmann, 1983).

Chromosome aberration assay

For the aberration assay, three different doses, including IC_{50} values as maximum dose, were prepared and separately added to 3-day old cultures (approximately 10^5 cells/60 mm dish). In the absence of metabolic activation, cultures were treated for 24 hours with the test article, while in the presence of metabolic activation, cells were treated for 6 hours because of its toxicity of S-9 and then maintained for 18 hours in the fresh medium i.e. to adjust a time equivalent to about 1.5 normal cell cycle lengths. Treatment was followed by addition of medium containing colcemid at a concentration of 0.2 μg/ml. After 2 hr further incubation in the presence of colcemid, metaphase cells were harvested by centrifugation and trypsinization. The cells were swollen with hypotonic (0.075 M) KCl solution for 20 min at 37°C, and washed three times in ice-cold fixative (methanol : glacial acetic acid = 3 : 1). After centrifugation, the fixative was removed, and cell pellet suspensions were prepared by pipetting gently. A few drop of cell pellet suspension were dropped onto precleaned glass microscope slides, and air dried. Slides were stained with 5% Giemsa buffered solution at pH 6.8 for scoring of chromosome aberrations. The number of cells with chromosomal aberrations was recorded on 100 well-spread metaphases at the magnification of 1,000 with Axioscope microscope (Karl Zeiss, FRG). The types of aberration were followed by JEMS-MMS [1988] classification. Breaks less than the width of a chromatid were designated as gaps in our criteria, and not included as chromosomal aberration. The incidence of polyploid and endoreduplicated cells was also recorded when these events were observed. Solvent-treated cells served as controls in this experiment.

Evaluation

CHL cells usually have less than 3.0% cells with spontaneous chromosome aberrations. Aberration frequencies, defined as aberrations observed divided by number of cells counted, were analyzed using Fisher's exact test (Altman, 1993) with Dunnett's adjustment and compared with results from the solvent controls. Therefore, data from count up well-spread 100 metaphase cells were expressed as percentages, and then dose-dependent response and the statistical significance in *p*-value will be considered as positive results in our judgement.

Results and Discussion

It has been widely assumed that mutation represents at least one step in carcinogenesis. The evidence supporting this idea is that mutagens are carcinogens [McCann *et al.*, 1975] and, for at least some compounds, mutagenic potency is closely correlated with carcinogenic potency [Meselson and Russel, 1977]. Moreover, mutagens and certain non-mutagenic carcinogens have also been found to induce chromosomal rearrangement [Zimmermann, 1971] which may affect carcinogenesis by altering gene expression, perhaps by allowing the activation of cellular cancer genes [Radman *et al.*, 1982].

Cytogenetic studies on mammalian cells *in vivo* as well as *in vitro* have been introduced as a screening method for DNA attacking substances. Several short term methods have been developed (Ames *et al.*, 1973; Maron and Ames, 1983; Mersch-Sundermann *et al.*, 1991) for predicting the carcinogenicity of chemicals and also been introduced for the evaluation of genotoxicity (Ishidate and Odashima, 1977; Matsuoka *et al.*, 1979; Radman *et al*, 1982; Hayashi *et al*, 1982, 1990, 1992; Ryu *et al.*, 1993a, 1994a, 1994b, 1996) and of antimutagenicity (Sato *et al.*, 1991; Ryu *et al.*, 1993b).

Taxol is a well known compound for the treatment of some types of human cancer. However, there was no report concerning genetic toxicity of taxol except Tinwell and Ashby's report (1994) which was recently published. For the chromosomal aberration assay of taxol and DAB in chinese hamster lung cells, 50% cell growth inhibition concentration was determined by MTT assay. The IC_{50} values of taxol and DAB were $1/16\times10^{-4}$ M (5.34 μg/ml) and 1×10^{-2} M (560 μg/ml), respectively. It means that the cytotoxicity of taxol revealed 100 times more cytotoxic than DAB in CHL cell line. The DMSO negative control is revealed only one percent spontaneous chromatid breakage in CHL cells. And also, the positive controls,

mitomycin C (0.1 μg/ml) for without metabolic activation and benzo(a)pyrene (200 μg/ml) for with metabolic activation, induced good chromosome aberrations in CHL cells as shown in Table 1 and 2.

In the highest concentration of taxol estimated as IC_{50} value ($1/16\times10^{-4}$ M), however, there was very rare metaphase cells at this concentration, so we lowered the highest concentration as $1/128\times10^{-4}$ M (0.67 μg/ml) for the study without metabolic activation system. The highest concentration (0.67 μg/ml) of taxol revealed nine percent chromosomal aberration with statistical significance in the absence of metabolic activation system, however, the strong positive results were not observed at the other dose-ranges used (Table 1). No statistical significant chromosomal aberration observed in the concentration range from 1. 34 μg/ml to 5.34 μg/ml in the presence of metabolic activation.

In *in vitro* chromosomal aberration assay, it is usually adopted the highest concentration as the IC_{50} value in the cell line used. However, it is not well understood why the metaphase cells were rare at the 5.34 μg/ml of taxol regardless the highest concentration as IC_{50} value used in this experiment.

In the case of DAB, no significant clastogenicity was not observed in the concentration range from 70 μg/ml to 280 μg/ml in the presence and absence of rat liver S9 metabolic activation system as shown in Table 2. However, in the 280 μg/ml of DAB without metabolic activation, we also cannot scored chromosome aberrations because of its presence of very rare metaphase cells.

It is easily assumed that the hydrolysis at C-10 and/or C-13 of taxol will be occurred in the presence of rat liver metabolic activation system (Fig. 1). In this respect, it is observed that

Table 1. The frequency of chromosome aberrations induced by taxol in chinese hamster lung fibroblasts

Treatment	Concentration (μg/ml)	With(+) or Without(-) S9 Mix	Chromosome aberrations/100 cells					Judgement
			chromatid		chromosome		Total aberration	
			breakage	exchange	breakage	exchange		
DMSO	-	-	1				1	
Taxol	0.67	-	8			1	9*	
	0.34	-	4				4	+
	0.17	-	1				1	
MMC	0.1	-	11	20		1	32	
DMSO	-	+	1				1	
Taxol	5.34	+	1			1	2	
	2.67	+	1				1	-
	1.34	+	1				1	
B[a]P	200	+	6	14		3	23	

DMSO : Dimethylsulfoxide, MMC : Mitomycin C, B(a)P : Benzo(a)pyrene *statistical singificance

Table 2. The frequency of chromosome aberrations induced by 10-deacetyl baccatin III in chinese hamster lung fibroblasts

Treatment	Concentration (μg/ml)	With(+) or Without(-) S9 Mix	Chromosome aberrations/100 cells					Judgement
			chromatid		chromosome		Total aberration	
			breakage	exchange	breakage	exchange		
DMSO	-	-	1				1	
10-Deacetyl	140	-	3				3	
baccatin III	70	-		1			1	-
MMC	0.1	-	4	9		2	15	
DMSO	-	+	1				1	
10-Deacetyl	280	+	1				1	
baccatin III	140	+					0	-
	70	+	1				1	
B[a]P	200	+	6	14		3	23	

DMSO : Dimethylsulfoxide, MMC : Mitomycin C, B(a)P : Benzo(a)pyrene

the cytotoxicity of taxol in the presence of S-9 fraction revealed about 10 times less toxic than that of taxol in the absence of S-9 (Table 1). So it is suggested that cytotoxicity of taxol will be lowered by the influence of drug-metabolizing enzyme system *in vivo*.

Recently, several new methods for the detection of genetic damages *in vitro* and *in vivo* based on molecular biological techniques were introduced according to the rapid progress in cellular and molecular toxicology. Among these methods, the single cell gel electrophoresis (comet assay) which can be detected DNA damages in cell level (Mckelvey-Martin *et al.*, 1993; Singh *et al.*, 1994), mouse lymphoma thymidine kinase gene assay (Clive *et al.*, 1983; Sawyer *et al.*, 1985), FISH (fluorescence *in situ* hybridization) (Hayashi *et al.*, 1994), PRINS (primed *in situ* hybridization) (Abbo *et al.*, 1993) and transgenic animal and cell line model as a parameter of *lac I* (Big Blue) (Kohler *et al.*, 1991) or *lac Z* (Muta Mouse) (Suzuki *et al.*, 1993) gene mutation are newly introduced into chemical toxicity evaluation. Also, *in vivo* supravital micronucleus assay by using acridine orange fluorescent staining (Hayashi *et al.*, 1990, 1992) was introduced instead of mouse bone marrow micronucleus assay. Our laboratory is now under progress these assays to evaluate and to elucidate the mechanism of genetic toxicity and/or carcinogenesis, and will be presented in near future for the genetic toxicity of taxol and its related compounds.

Acknowledgement

The authors thank to professor Byung-Soo Kim, department of applied statistics, college of business and economics, Yonsei university for his statistical analysis.

References

Abbo, S., R.P. Dunford, T.E. Miller, S.M. Reader, and I.P. King (1993) : Primer-mediated *in situ* detection of the B-hordein gene cluster on barley chromosomes 1H. Genetics **90:** 11821-11824.

Altman, D.G. (1993) : Practical statistics for medical research. In Chapter 10 : "Comparing groups-categorical data." London: Chapman & Hall. pp.229-276.

Ames, B.N., W.E. Durston, E. Yamasaki and F.D. Lee, (1973) : Carcinogens are mutagens : a simple test system combining liver homogenates for activation and bacteria for detection, Proc. Natl. Acad .Sci., USA, **70:** 2281-2285.

Ames, B.N., J. McCann, and E. Yamasaki, (1975) : Method for detecting carcinogens and mutagens with *Salmonella/* mammalian-microsome mutagenicity test, Mutation Res., **31:** 347-364.

Bissett, D. and S.B. Kaye, (1993) : Taxol and taxotere-current status and future prospects. Eur. J. Cancer, **29:** 1228-1231.

Burkhart, C.A., J.W. Berman, C.S. Swindell, and S.B. Horwitz, (1994) : Relationship between the structure of taxol and other taxanes on induction of tumor necrosis factor-α gene expression and cytotoxicity. Cancer Res., **54:** 5779-5782.

Clive, D., R. McCuen, J.F.S. Spector, C. Piper and K.H. Mavournin (1983) : Specific gene mutations in L5178Y cells in culture : A report of the U.S. Environmental Protection Agency Gen-Tox Program. Mutation Res., **115:** 225-251.

Hayashi, M., T. Sofuni and M. Ishidate, Jr (1982) : High sensitivity in micronucleus induction of mouse strain. Mutation Res., **105:** 252-256.

Hayashi, M., T. Morita, Y. Kodama, T. Sofuni and M. Ishidate, Jr (1990) : The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides. Mutation Res., **245:** 245-249.

Hayashi, M., Y. Kodama, T. Awogi, T. Suzuki, A.O. Asita, and T. Sofuni (1992) : The micronucleus assay using peripheral blood reticulocytes from mitomycin C- and cyclophosphamide-treated rats. Mutation Res., **278:** 209-213.

Hayashi, M., J. Maki-Paakkanen, H. Tanabe, M. Honma, T. Suzuki, A. Matsuoka, H. Mizusawa and T. Sofuni, (1994) : Isolation of micronuclei from mouse blood and fluorescence in situ hybridization with a mouse centromeric DNA probe. Mutation Res., **307:** 245-251.

Horwitz, S.B. (1992) : Mechanism of action of taxol. TIPS, **13:** 134-137.

Ishidate, M. and S. Odashima, (1977) : Chromosome test with 134 compounds on chinese hamster cells in vitro-A screening for chemical carcinogens. Mutation Res., **48:** 337-354.

Jederny, J., R.A. Walk, U. Hackenberg and G. Rohrborn (1988) : Chromosomal abnormalities and sister-chromatid exchange in bone marrow cells of mice and chinese hamsters after inhalation and intraperitoneal administration. Mutation Res., **203:** 1-10.

JEMS-MMS (1988) : Atlas of chromosome aberration by chemicals, Japanese Environmental Mutagen Society-Mammalian Mutagenicity Study Group, Tokyo, Japan (in Japanese) Kingston, D.G.I., (1991) : The chemistry of taxol. Pharmac. Ther., **52:** 1-34.

Kingston, D.G.I., G. Samaranayake and C.A. Ivey, (1990) : The chemistry of taxol, a clinically useful anticancer agent. J. Natural Products, **53:** 1-12.

Kohler, S.W., G.S. Provost, A. Fieck, P.L. Kretz, W.O. Bullock, J.A. Sorge, D.L. Putman and J.M. Short (1991) : Spectra of spontaneous and mutagen-induced mutations in the *lac I* gene in transgenic mice. Proc. Natl. Acad. Sci. USA, **88:** 7958-7962.

Lahdetie, J., A. Keiski, A. Suutari, and J. Toppari, (1994) : Etoposide (VP-16) is a potent inducer of micronuclei in male rat meiosis : spermatid micronucleus test and DNA flow cytometry after etoposide treatment. Environ. Mol. Mutagen., **24:** 192-202.

Maron, D.M. and B.N. Ames, (1983) : Revised methods for the *Salmonella* mutagenicity test. Mutation Res., **113:** 173-215.

Matsuoka, A., M. Hayashi and M. Ishidate (1979) : Chromosomal aberration test on 29 chemicals combined with S-9 mix *in vitro*. Mutation Res. **66:** 277-290.

McCann, J., E. Choi, E. Yamasaki and B.N. Ames, (1975) : Detection of carcinogens as mutagens in the *Salmonella*/ microsome test : assay of 300 chemicals. Proc. Natl. Acad. Sci. USA, **72:** 5135-5139.

Mersch-Sundermann, V.S., S. Kerekordes and S. Mochayedi, (1991) : Sources of variability of the *Escherichia coli* PQ 37 genotoxicity assay (SOS chromotest). Mutation Res., **252:** 51-60.

Mckelvey-Martin, V.J., M.H.L. Green, P. Schemezer, B.L. Pool-Zobel, M.P. De Meo and A. Collins, (1993) : The single cell gel electrophoresis assay (comet assay): A european review. Mutation Res., **288:** 47-63.

Meselson, M. and K. Russell, (1991) : Comparison of carcinogenic and mutagenic potency. In : Hiatt, H.H., Watson, J.D., Winstend, J.A. (eds.) : "Origin of Human Cancer". Cold Spring Harbor Laboratory, pp.1473-1481.

MOE report (1992) : "Toxicity evaluation of synthetic chemicals (V)". Ministry of Environment, Seoul, Korea, pp. 19-63.

MOE report (1993) : "Toxicity evaluation of synthetic chemicals (VI)". Ministry of Environment, Seoul, Korea, pp. 53-128.

MOE report (1994) : "Toxicity evaluation of synthetic chemicals (VII)". Ministry of Environment, Seoul, Korea, pp. 29-129.

MOE report (1995) : "Toxicity evaluation of synthetic chemicals (VIII)". Ministry of Environment, Seoul, Korea, pp. 61-85.

Mosmann, T., (1983) : Rapid colorimetric assay for cellular growth and survival : Application to proliferation and cytotoxicity assays. J. Immunol. Methods, **65:** 55-63.

OECD (1993) : "OECD giudeline for the testing of chemicals, Documents 473, Genetic toxicology : *in vitro* mammalian cytogenetic test". Organization for Economic Co-operation and Development, Paris, France.

Parness, J., D.G.I. Kingston, R.G. Powell, C. Harracksingh and S.B. Horwitz, (1982) : Structure-activity study of cytotoxicity and microtubule assembly *in vitro* by taxol and related taxanes. Biochem. Biophys. Res. Commun., **105,** 1082-1089.

Radman, M., P. Jeggo and R. Wagner, (1982) : Chromosomal rearrangement and carcinogenesis. Mutation Res., **98:** 249-264.

Rowinsky, E.K. and R.C. Donehower, (1991) : The clinical pharmacology and use of antimicrotubule agents in cancer chemotherapeutics. Pharmac. Ther., **52:** 35-84.

Ryu, J.-C., S. Lee, K.-R. Kim, M. Kim, I.-M. Chang and J. Park (1993a) : A study on the clastogenicity of trichothecene mycotoxins in chinese hamster lung cells. Korean J. Toxicol., **9**(1) : 13-21.

Ryu, J.-C., S.-H. Kim, K.-R. Kim, S. Lee, M. Kim, H.-K. Jung and J. Park (1993b) : A study on the antimutagenic effect of cinnamic acid derivatives in *Escherichia coli* PQ 37 (SOS Chromotest) (I). Environ. Mutagens & Carcinogens, **13**(1) : 8-17.

Ryu, E.-K., K.-R. Kim, S. Lee, J. Park and J.-C. Ryu, (1994a) : Evaluation of the genetic toxicity of synthetic chemicals, chromosomal aberration test with 28 compounds in chinese hamster lung cells *in vitro*. The Fall Conference of the Korean Society of Toxicology and the Korean Environmental Mutagen Society, pp. 119.

Ryu, J.-C., S. Lee, K.-R. Kim and J. Park, (1994b) : Evaluation of the genetic toxicity of synthetic chemicals (I). Chromosomal aberration test on chinese hamster lung cells *in vitro*. Environ. Mutagens & Carcinogens, **14**(2): 138-144.

Ryu, J.-C., K.-R. Kim, E.-K. Ryu, H.-J. Kim, S.-Y. Lee, O.-S. Kwon, J. Park and I.-M. Chang, (1996) : Evaluation of the genetic toxicity of synthetic chemicals (III). Chromosomal aberration assay with 28 chemicals in chinese hamster lung cells *in vitro*, Environ. Molecul. Mutagenesis (submitted)

Sato, T., K. Chikazawa, H. Yamamori, Y. Ose, H. Nagase and H. Kito, (1991) : Evaluation of the SOS chromotest for the detection of antimutagens. Environ. Mol. Mutagenesis, **17:** 258-263.

Sawyer, J., M.M. Moore, D. Clive and J. Hozier, (1985) : Cytogenetic characterization of the L5178Y $TK^{+/-}$ 3.7.2C mouse lymphoma cell line. Mutation Res., **147:** 243-253.

Schiff, P.B., J. Fant and S.B. Horwitz, (1979) : Promotion of microtubule assembly *in vitro* by taxol, Nature, **277:** 665-667.

Singh, P.N., R.E. Stephens and E.L. Schneider, (1994) : Modification of alkaline microgel electrophoresis for sensitive detection of DNA damage. Int. J. Radiation Biol.,

66(1) : 23-28.

Suzuki, T., M. Hayashi, T. Sofuni and B.C. Myhr, (1993) : The concomitant detection of gene mutation and micronucleus induction by mitomycin C *in vivo* using *lac* Z transgenic mice. Mutation Res., **285:** 219-224.

Tinwell, H. and J. Ashby, (1994) : Genetic toxicity and potential carcinogenicity of taxol. Carcinogenesis, **15:** 1499-1501.

Wani, M.C., H.L. Taylor, M.E. Wall, P. Coggon and A.T. McPhail, (1971) : Plant antitumor agents, VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. JACS, **93:** 2325-2327.

Wiernik, P.H., E.L. Schwartz, J.J. Strauman, J.P. Dutcher, R. B. Lipton and E. Paietta, (1987) : Phase I clinical and pharmacokinetic study of taxol. Cancer Res., **47:** 2486-2493.

Zimmermann, F.K. (1971) : Induction of mitotic gene conversion by mutagens. Mutation Res., **11:** 327-337.

Chinese hamster lung cell을 이용한 Taxol과 10-deacetyl baccatin III의 *in vitro* 염색체 이상 시험연구

류재천, 김경란, 류은경, 김현주, 권오승, 송충의*, 마응천, 장일무****

한국과학기술연구원 도핑콘트롤센터, 응용과학연구부*, 서울대학교 천연물과학연구소**

적 요

항암제로 널리 쓰이는 **Taxol**에 관하여 최근에 **in vivo** 소핵실험에서 매우 강한 소핵유발능을 나타낸다는 보고 **(Carcinogenesis, 15, 1499-1501, 1994)**에 따라 **Chinese hamster lung cell**을 이용하여 **in vitro**에서 염색체이상의 유발 유무를 검정하고, **in vivo**에서의 강한 소핵유발능이 **Taxol** 분자구조의 어느 **moiety**에 의한 것인가를 확인하고자 **Taxol** 생합성의 전구체로 널리 쓰이는 **10-deacetyl baccatin III**와 병행 비교하여 대사활성화의 관여 여부를 확인하고자 하였다.

그 결과 **Taxol**은 **10-deacetyl baccatin III**에 비해 약 **100**배 정도의 세포독성을 **Chinese hamster lung cell**에서 나타내었고, 염색체이상시험에서는 **Taxol**의 경우, 대사 활성 부재하에서는 약한 양성을 보였으나, 대사활성 존재하에서는 염색체 이상 유발 유무를 관찰할 수 없었고, **10-deacetyl baccatin III**는 대사 활성화 존재 및 부재하 모두에서 강한 염색체 이상유발능은 관찰할 수 없었다. 더불어 생체내 대사효소에 의한 **Taxol**의 대사가 **+S-9**과 **-S-9**에 의한 세포독성 **data**에서도 유추할 수 있었으나 **10-deacetyl baccatin III**는 대사활성 존재 및 부재 모두에서, **Taxol**은 대사활성 존재하에서만 염색체이상 음성의 결과를 보여 주고, 대사활성 부재하에서는 약한 양성을 보여, **Taxol**의 염색체 이상 유발능은 **C-13**의 **ester**결합기의 관여에 의한 유전독성으로 사료된다.