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Cytogenetic Effects of Chloramphenicol in Rats

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ABSTRACT. Chromosomal aberration in bone marrow cells of albino rat (*Rattus norvegicus*) were taken as an indication of genotoxic action of chloramphenicol (CAP): Young adult male rats (*Rattus norvegicus*) received oral administration of 50 and 100 mg/kg of CAP. Samples of bone marrow cells were taken at 6, 12, 18, 24 and 30 hours post treatment. Pregnant female rats received a similar treatment at midterm and later stages of pregnancy in order to examine the genotoxic effects of CAP (50 mg/kg) on embryos through transplacental exposure. Chloramphenicol did not induce any effects after 50 mg/kg but chromosomal aberration were recorded at 100 mg/kg at 12 and 18 hours post treatment and 24 hours later the number of aberrent cells returned to control values in both young adult male and newborn pups of treated pregnant female rats.

Chloramphenicol (CAP) is an antibiotic agent widely used in human practice. However, it has various side effects.

The international agency for cancer research has classified this compound as a drug with limited evidence of carcinogenicity in humans (IARC 1982). From the genetic point of view, mutational assays showed that CAP is not mutagenic for various strains of *Salmonella typhimurium* and *E. coli* sp. Negative results were also obtained in *Analidopsis* and in *Drosophila melanogaster*. In contrast, CAP can cause chromosomal aberrations in plant, rodent, and human somatic cells and this seems to be the only genetic effect of the drug (Rosenkranz 1988). Chloramphenicol is an

inhibitor of bacterial protein synthesis; it binds to the large subunit of the bacterial ribosome, thereby blocking peptide transfer (Davis *et al.* 1988). Chloramphenicol inhibits eukaryotic protein synthesis (Weisberger *et al.* 1963, Weisberger and Wolfe 1964). DNA replication inhibition has been also reported in mammalian cells (Yunis and Harrington 1960, Yunis *et al.* 1972, Murray *et al.* 1983). The present work is an attempt to examine the cytogenetic effects of CAP on adult animals and transplacentally exposed embryos in order to understand the mechanism by which this drug may/or may not affect dividing mitotic cells.

Materials and Methods

Experiment I:

Fifty five, (six to eight weeks old young, male rats) weighing 120 ± 20 gm were placed into three groups. The first group of five rats was left as control and given only sodium succinate by a metallic stomach tube. The second and the third groups 25, animals each, were orally administered 50 and 100 mg/kg CAP, respectively, dissolved in sodium succinate (4.8 mg/ml) using a metallic stomach tube. Five animals were killed for each fixation time 6, 12, 18, 24 and 30 hours post-treatment; two hours before killing, the animals were injected intraperitoneally with 0.5 cc of 0.04% solution of colchicine. Metaphase chromosomes were obtained from femoral bone marrow cells, that were flushed in phosphate buffer solution. The suspension was centrifuged for 10 minutes at 500 rpm. The supernatant fluid was discarded and the pellet of cells was resuspended in a hypotonice solution (0.075 kcl) and kept at 37°C for 20 minutes. Cell suspensions were centrifuged for 10 minutes and the supernatant was removed and the cells were fixed in methyl acetic acid for at least 20 minutes. Then the cells were spread on cooled slides and stained by Giemsa stain. For each animal, 50 metaphases were examined microscopically using a 10X eye piece and 100X oil immersion objective lens and photomicrographs were taken whenever necessary.

Experiment II:

Virgin females were mated and the day on which sperms were found in the vaginal smear was designated as day 0 of gestation. The pregnant females were given orally similar doses of CAP, by using a metallic stomach tube daily for 10 successive days from 8th to 18th day of gestation. Control pregnant females rats were treated by solvent (sodium succinate). Three pregnant females for each dose were used. After delivery, 2 newborn pups were chosen randomly from each female. Metaphase chromosomes were obtained from femoral bone marrow cells as in

experiment I; 50 metaphases were examined blindly for each newborn. Chromosomal aberrations were detected and photomicrographs were taken whenever necessary.

Statistical Analysis:

Data were statistically analysed using Student "t" test (Snedecor 1969).

Results

In the 1st experiment, animals were treated with an oral dose of CAP in either 50 or 100 mg/kg. The obtained results showed that CAP (50 mg/kg) did not cause any significant differences between the frequencies of aberrent cells observed in treated and untreated animals. However, at a dose of 100 mg/kg, aberrent metaphases were greater than those obtained with 50 mg/kg and were highly significant compared to control values after 18 hours (Table 1). Most of the aberration detected were dicentric chromosomes, exchange figures and chromosomal breaks, (Fig. 2) 18 hours after treatment. There was a decline in the incidence of aberrations to control values by 30 hours (Fig. 1).

In the 2nd experiment, pregnant females received the same treatment as a daily dose from day 8 to day 18 of gestation. Embryos were exposed transplacentally to CAP. Three days after delivery the pups were taken and examined for chromosomal damage. No significant changes were seen at a dose of 50 mg/kg (2.3%) compared to control values (0.7%). Following the high dose, 100 mg/kg, a highly significant increase ($P < 0.001$) was recorded; the frequency of aberrations reached 9% (Table 2). From the obtained results, we can conclude that CAP is a genotoxic drug on embryos and its genotoxicity on embryos is higher than that on the adult animals.

Discussion

Present results showed that CAP had somewhat limited effects in inducing chromosomal damage in bone marrow cells in adult rats. Although slight increases in chromosomal aberrations were seen 12 and 18 hours post treatment, significant changes were noted only at 24 and 30 hours post treatment. These results are in agreement with other studies that have reported a limited mutagenicity for CAP (Mitus and Coleman 1970, Kong-oo 1979). Recently Isabella *et al.* (1990) concluded that CAP acts as a radiomimetice agent directly producing double strand breaks, after treatment lasting for a whole cell cycle. Moreover, they reported that CAP

Table 1. Chromosomal damage in bone marrow cells of treated male rats with chloramphenicol

(Mean ± S.E.)

Fixation time (hour)	Treat-ment mg/kg	No. of examined rat	Total cell scored	Chromosomal damage			Total damage		
				Dicentric	Exchange figures	Breaks	No.	%	M ± S.E
Control	–	5	250	3	–	–	3	1.2	0.6 ± 0.4
6	50	5	250	3	–	–	3	1.2	0.6 ± 0.4
12		5	250	3	2	1	6	2.4	1.2 ± 0.58
18		5	250	2	2	–	4	1.6	0.8 ± 0.37
24		5	250	3	–	–	3	1.2	0.6 ± 0.24
30		5	250	3	–	–	3	1.2	0.6 ± 0.0
6	100	5	250	3	1	–	4	1.6	0.8 ± 0.58
12		5	250	6	3	2	11	4.2	2.2 ± 0.58
18		5	250	9	2	3	14	5.6	*2.8 ± 0.73
24		5	250	5	1	1	7	2.8	1.4 ± 0.75
30		5	250	3	–	–	3	1.2	0.6 ± 0.4

*($P < 0.05$)

Table 2. Effect of chloramphenicol on chromosomal damage of embryo during pregnancy through transplacental exposure

(Mean ± S.E.)

Treatment (hour)	No. of pregnant female	No. of newly born	Total cell examined	Chromosomal damage			Total damage		
				Dicentric	Exchange figures	Breaks	No.	%	M ± S.E
Control	3	6	300	2	–	–	2	0.7	0.3 ± 0.21
50	3	6	300	3	2	2	7	2.3	1.7 ± 0.48
100	3	6	300	12	8	7	27	9.0	***4.5 ± 0.62

***($P < 0.01$)

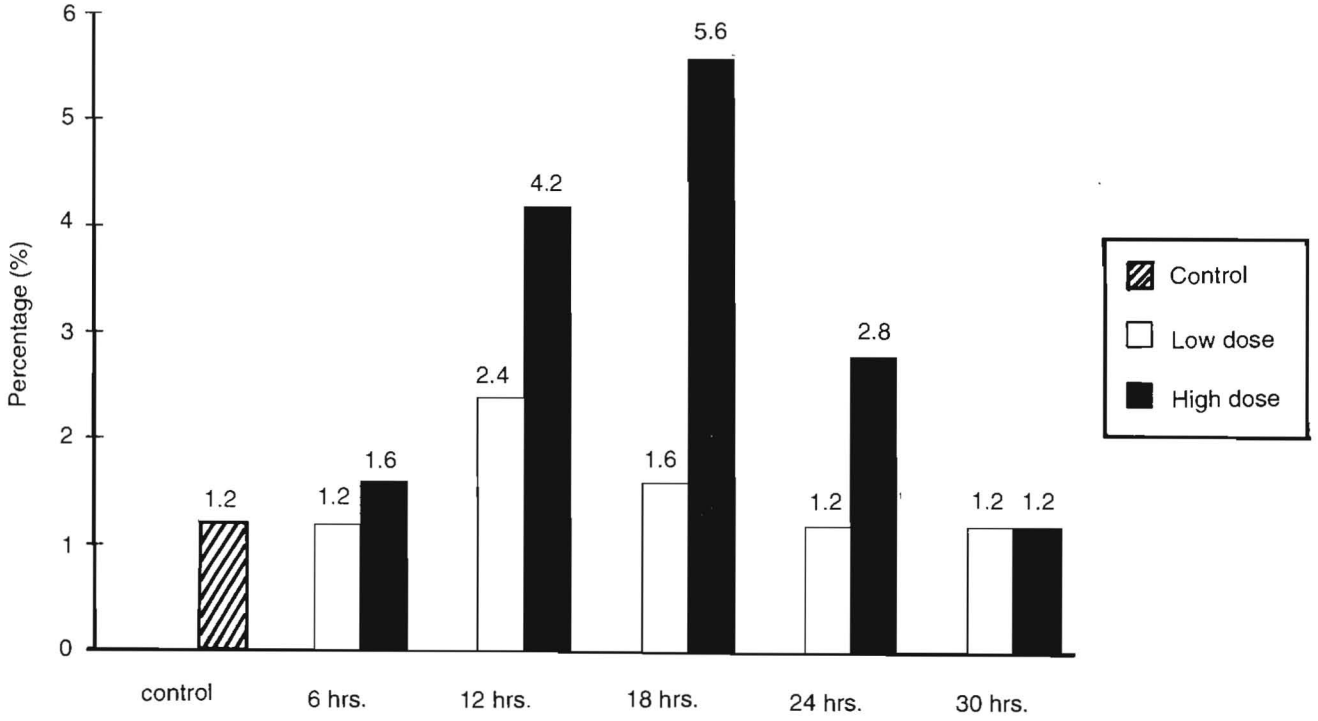

Fig. 1. Percentage of chromosomal damage in metaphases of male rats treated by chloramphenicol.

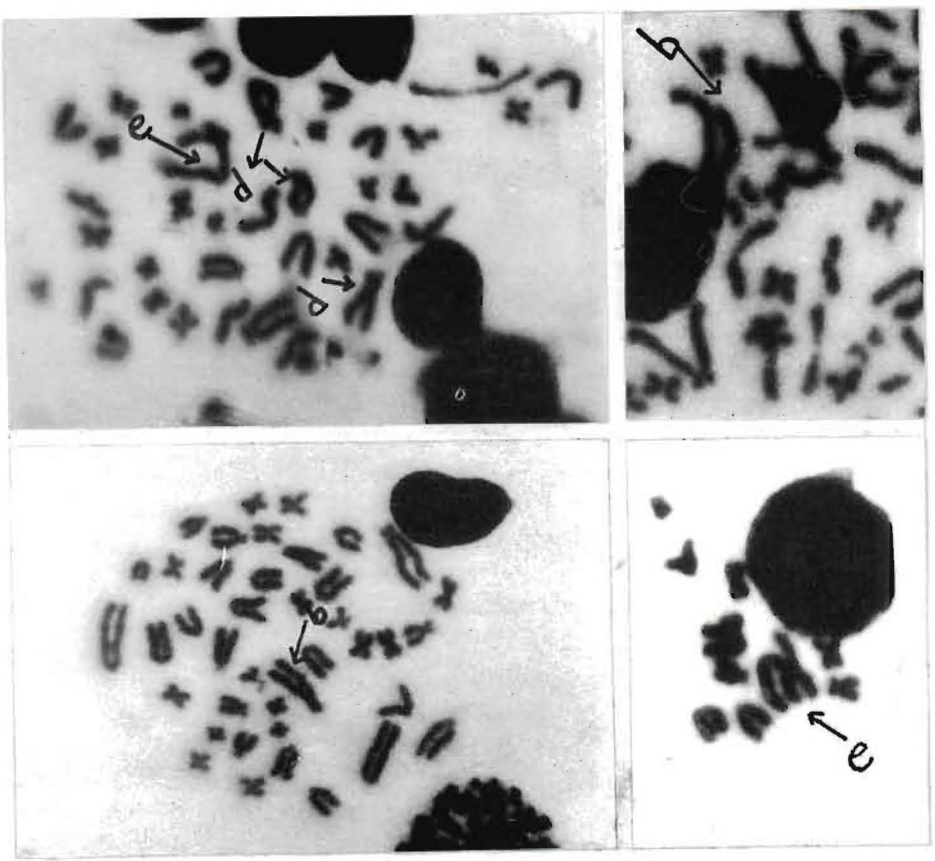

Fig. 2. Photomicrographs of Dicentric chromosomes (d) exchange figures (e) and chromosomal breaks (b) from metaphases of male rats treated with chloramphenicol.

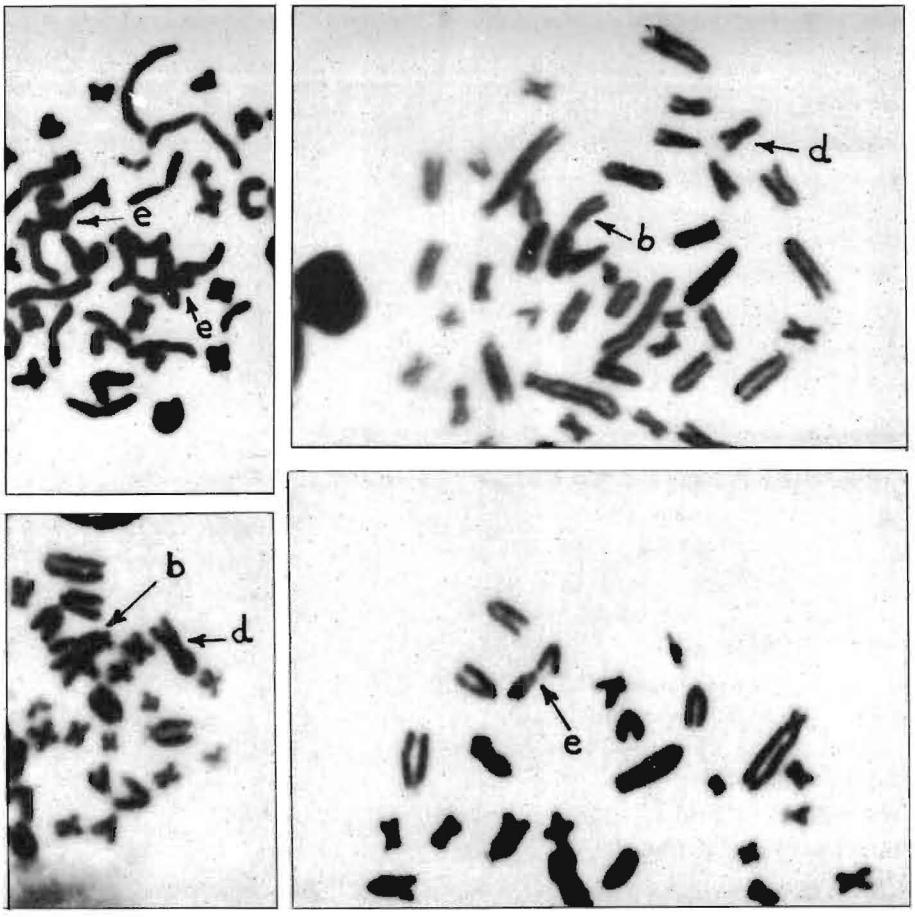

Fig. 3. Photomicrographs of Dicentric chromosomes (d), exchange figures (e) and chromosomal breaks (b) from metaphases of newborns rat to treated pregnant mother rat with chloramphenicol.

produced only chromatid type aberration vacuoles due to its effect on chromosome condensation. The same CAP concentration was able to inhibit cell proliferation by inhibition of DNA synthesis (Rainaldi *et al.* 1984). In this study, the most common aberrations have been dicentric chromosomes, exchange figures and chromatid breaks, chloramphenicol action in G2 phase of mitotic division. Chloramphenicol has been also shown to induce either breaks or exchange when administered in G2 phase after treatment with 8 ethoxycaffeine (Nuti and Buiatti 1967) or between G1 and G2 (Wolff 1960).

The formation of exchange type aberrations which require DNA synthesis to be performed even shows that. CAP acts by affecting DNA replication (Kihlaman and Natarjan 1984). A lack of a significant increase of aberrations after 24 and 30 hours, post treatment emphasizes that treatment with CAP at high doses for a short periods is unable to cause genotoxicity of metaphases. This indicates that CAP did not produce direct or indirect interference with DNA replication (Yunis and Harrington 1960, Yunis *et al.* 1972, Murray *et al.* 1983). They also recorded that the prolonged treatment with CAP did not elicit any effect on DNA replication.

Data obtained from bone marrow cells of newborn rat that had received CAP through transplacental exposure during pregnancy showed that CAP treatment had a more potent effect on embryonic mitotic dividing cell than on adult chromosomes. These data indicate that CAP is genotoxic on embryonic chromosomes at midterm and later stages of pregnancy and the most aberrations were dicentric chromosomes (Fig. 3). CAP may have an effect on the amount of crossing over between some types of chromosomes during mitotic cell division. An addition, other aberrations such as exchange figures and breaks showed that CAP interferes with cell division by inhibition. The cytogenetic studies of CAP on embryonic chromosomes remain very rare. But other studies of CAP on embryos were recorded by (Nishimura and Katani 1970, Rajchgat *et al.* 1982). They found that CAP genotoxicity on embryos due to high degree of placental transfer, accumulation of CAP in maternal serum and decreased hepatic conjugation; after delivery, the infant received CAP excreted in breast milk. Decreased hepatic conjugation of CAP by pregnant females may result in toxic accumulation of CAP and increased maternal serum concentration ratio (Plomp *et al.* 1983 and Mulhall *et al.* 1983).

References

Davis, B.D., Dulbecco, R., Eisen, H.N. and **Ginsberg, H.S.** (1988) *Microbiology* 3rd **(ed.)** Harper and Row, New York.

IARC (1982) Monographs on the evaluation of the carcinogenic risk of chemicals to human chemicals, Industrial Processes and Industries Associated with cancer to humans IRAC Monographs Supp. 4, *International Agency for research on Cancer,* Lyon.

Isabella, S., Sofia, C., Giuseppe, R. and **Nicola, L.** (1990) Induction of chromosomal aberration and SCE by chloramphenicol. *Mutation. Res.*, **248:** 145-153.

Kihlman, B.A. and **Natarjan, A.J.** (1984) Potentiation of chromosomal aberration by inhibitors of DNA repair. *In:* **Collins, A.R.S.** *et al.* **(Eds)** DNA repair and its inhibition towards an analysis of mechanism Nucleic acids symposium. *Ser. IRL* Press, Oxford, **13:** 319-339.

Kong-oo, G. (1979) Chloramphenicol and chromosomal morphology, *J. Med.*, **10:** 159-166.

Mitus, W.J. and **Coleman, N.** (1970) *In vitro* effect of chloramphenicol on chromosomes. *Blood,* **35:** 689-694.

Mulhall, A. de Louvios J. and **Hurley, R.** (1983) Chloramphenicol toxicity in neonates: its incidence and prevention. *Br. Med. J.* **287:** 1424-1427.

Murray, T., Dawney, K.M. and **Yunis, A.A.** (1983) Chloramphenicol mediated DNA damage and its possible role in the inhibitory effects of chloramphenicol on DNA synthesis. *J. Lab. Clin. Med.*, **102:** 926-932.

Nishimura, T. and **Katani, Y.** (1970) Special characteristics of absorption and excretion of chloramphenicol in premature and excretion of chloramphenicol in premature and newborn infants. *Bull/Osaka Med. Sch.*, **16:** 169-181.

Nuti Ronchi, V. and **Buiatti, M.** (1967) The effect of chloramphenicol on the chromosome breaking action of 8-ethoxycaffeine and 6-methylacaumarin in Vicia faba. *Mutation Res.*, **4:** 615-619.

Plomp, T.A., Thiery, M. and **Maes, R.A.A.** (1983) The passage of thiamphenicol and chloramphenicol into human milk after single and repeated oral administration. *Vet. Hum. Toxicol.*, **25:** 169-172.

Rainaldi, G., Sessa, M.R. and **Mariani, T.** (1984) Inhibitors of DNA synthesis induce sister chromatid exhange at the early S. phase of the cell cycle. *Chromosom,* **90:** 86-90.

Rajchgat, P., Prober, G.C. and **Soldin, S.** (1982) Inhibition of chloramphenicol therapy in the newborn infant. *J. Pediatr.*, **101:** 1018-1021.

Rosenkranz, H.S. (1988) Chloramphenicol magic bullet or double edge sword. *Mutation Res.*, **106:** 1-16.

Snedecor, G.W. (1969) *Statistical Methods.* Ames., Iowa, USA: The Iowa State University Press.

Weisberger, A.S. and **Wolfe, S.** (1964) Effect of chloramphenicol on protein synthesis. *Fed. Proc.*, **23:** 976-978.

Weisberger, A.S., Armentrout, S. and **Wolfe, S.** (1963) Protein synthesis by reticulocyte ribosomes: I. Inhibition of polyuridylic acid-induced ribosomal protein synthesis by chloramphenicol. *Proc. Natl. Acad. Sci.*, (USA) **50:** 86-89.

Wolff, S. (1960) Radiation studies on the nature of chromosome breakage. *Am. Natural,* **94:** 85-93.

Yunis, A.A. and **Harrington W.J.** (1960) Pallerns of inhibition by chloramphenicol of nucleic acid synthesis in human bone marrow and leukemic cells. *J. Lab. Clin. Med.*, **56:** 831-832.

Yunis, A.A., Manyan, D.R. and **Arimura, G.K.** (1972) Comparative effect of chloramphenicol and thiamphenicol on DNA and mitochondrial protein synthesis in mammalian cells. *J. Lab. Clin. Med.*, **81:** 713-718.

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التأثيرات الوراثية الخلوية للكلورامفينيكول (عامل ضد الميكروبات) في الفأر الأبيض

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استهدف هذا البحث تقنين الآثار الجانبية للمضاد الحيوي (كلورامفينيكول) على المستوى الجيني من خلال دراسة التشوه الكروموسومي .

وقد استخدم في هذا البحث مجموعتين من الفئران البيضاء (جنسي راتس نورفيجيكس) - المجموعة الأولى لذكور بالغة عولجت عن طريق الحقن الفمي بجرعتين من الكلورامفينيكول وهي الجرعات الآمنة لاستخدم الكلورامفينيكول كمضاد حيوي على الإنسان وهما : (٥٠ مجم لكل كجم من وزن الجسم ، ١٠٠ مجم لكل كجم من وزن الجسم) وتمت المعالجة ولوحظ التشوه الكروموسومي بعد ٦ ، ١٢ ، ١٨ ، ٢٤ ، ٣٠ ساعة .

وتبين من الفحص المجهري للكروموسومات من نخاع عظم الفأر ان الجرعة الصغرى (٥٠) مجم لكل كجم من وزن الجسم) لم تسجل انحرافاً معنوياً في التشوه الكرموسومي على مدى الأزمنة المختلفة .

بينما الجرعة الكبرى (١٠٠ مجم لكل كجم من وزن الجسم) سجلت

انحرافا معنويا عند الوقت ١٢ ، ١٨ وبدء الانحراف يقل مع زيادة الوقت .

أما المجموعة الثانية فكانت على الأجنة التي عولجت أمهاتها الحوامل بنفس الجرعات الآمنة التي عولجت بها الذكور البالغة .

وتمت المعالجة لمدة عشرة أيام متتالية ابتداء من اليوم الثامن حتى اليوم الثامن عشر من الحمل أي قبل الولادة بثلاث أيام وتبين من الفحص الكروموسومي للمواليد التي عولجت أمهاتها بالجرعات الآمنة - زيادة طردية ومعنوية على معدل التشوه الكروموسومي يتناسب مع الجرعات المستخدمة .