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INVESTIGATION OF CYTOGENETIC EFFECTS OF 2, 4, 5 TRIPHENYL IMIDAZOLE BY RAT BONE MARROW MICRONUCLEUS TEST

2, 4, 5 TRİFENİL İMİDAZOLUN SİTOGENETİK ETKİLERİNİN SIÇAN KEMİK İLİĞİ MİKRONÜKLEUS TESTİ İLE ARAŞTIRILMASI

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The cytogenetic effects of 2, 4, 5 triphenyl imidazole on the micronucleus formation were investigated in rats. Increasing doses of the substance were injected i.p. into Wistar rats and polychromatic and normochromatic erythrocytes were scored for the formation of micronuclei in bone marrow smears. The number of micronuclei was found about 2-3 times more in 2,4,5 triphenyl imidazole treated rats than the control groups. This increase in the formation of micronucleus indicates the clastogenic effects of the substance on rats and may have similar effects on other mammals and human population.

2,4,5 trifenil imidazolun sitogenetik etkileri memeli hayvanlarda mikronükleus oluşturması açısından araştırılmıştır. Bu madde artan dozlar halinde Wistar sıçanlara i.p. olarak verilmiş ve hazırlanan kemik iliği yayma preparatlarında, polikromatik ve normokromatik eritrositlerde meydana gelen mikronükleuslar sayılmıştır. Mikronükleus sayısı 2, 4, 5 trifenil imidazole maruz kalan sıçanlarda kontrol gruplarından 2-3 kat daha fazla bulunmuştur. Mikronükleus oluşumundaki bu artış bu maddenin sıçanlar üzerindeki klastojenik etkisini göstermektedir ve benzer etkileri bu ilaç hammaddesini kullanan diğer memeliler ve insanlar üzerinde gösterebilir.

Keywords: *Micronucleus; 2, 4, 5 triphenyl imidazole; Rat bone marrow.*

Anahtar Kelimeler: *Mikronükleus; 2, 4, 5 trifenil imidazol; Sıçan kemik iliği.*

Introduction

Chromosome structural aberrations, gene mutation and occurrence of aneuploidi are all involved in cancer development and inherited clinical disorders. To prevent human exposure to potential genotoxic inducers, it is important to identify such agents in mammalian systems (1).. The Micronucleus (MN) test is one the important *in vivo* cytogenetic screening assay for the detection of structural chromosomal damage induced by spindle poisons of clastogens in cells (2-7). The micronuclei are originated from chromosome breakages from lagging chromosome in anaphase that has not

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travelled to the appropriate pole of the spindle, to be included in the main nucleus of the daughter cell. This material is distributed to only one of the daughter cells in the following cell division (8).

A large number of studies have been done with various mutagenic agents using MN test in a variety of cells such as mouse or rat bone marrow (9, 10), mouse or rat peripheral blood (10-12), human lymphocytes (13-15); mussel gill cells (16), and plant cells (17).

Imidazole is a simple heterocyclic ring which is included in many complex organic compounds. After peroral administration, it is rapidly absorbed from the gastrointestinal tract and metabolised (18). A large number of imidazole derivatives have been synthesized and tested for their antihelmintic, antifungal, antibacterial, antienflamatuar and antihistaminic activities (19-21). Imidazole and its metabolites, hydantoin, hydantoic acid, N-acetyl-imidazole and histamin have been found nonmutagenic (22). Negative results have also been obtained for several alkyl-substituted imidazoles from a mutant *Klepsiella pneumoniae* tester strain (23). Moreover, 45 derivatives of nitroimidazoles have been found mutagenic and genotoxic in AMES test (24).

The purpose of the present work was to evaluate the incidence of micronuclei induced by 2, 4, 5 triphenyl imidazole in rat bone marrow cells after administration intraperitoneally at different doses. In this work, the clastogenic effects of 2, 4, 5 have been investigated triphenyl imidazole on bone marrow cells of rats.

Materials and Methods

2, 4, 5 triphenyl imidazole was provided from Işıkdağ, Uçucu, Çakır (Anadolu University, Faculty of Pharmacy, Turkey (25), dimethyl sulphoxide (DMSO) (Sigma), RPM 1640 medium (Sigma), May-Grunwald and Giemsa stain (Sigma), 2-acetamidofluorene (2-AAF) (Sigma) and Wistar rat from Laboratory Animal Section Faculty of Medicine, Osmangazi University, Eskişehir, Turkey.

Animals weighing 250-300 gr were maintained under standart conditions of temperature and humidity and were supplied with free access to standart food and fresh tap water. For the micronucleus assay, each of five doses of 2, 4, 5 triphenyl imidazole (1, 2, 4, 6 and 8 mg/ kg) dissolved in DMSO was injected i.p. with physiological saline to 10 animals (two animals for each doses). Treatment schedule was one administration at 24 h intervals for 7 days. For control, 10 separate animals received only DMSO and one rat was received nothing. All the animals were killed 24 h after medication. 2-AAF at a consantration of 150 mg/kg per animal was used as a positive control only at the 48 h sacrifice time (26).

Both femora were removed through the pelvic bone just below the knee as described by Henderson *et al.* (10). The femur bone was freed from extra muscles and cleared thoroughly. After bisecting the proximal end of the femur, bone marrow was flushed out with a syringe filled up 0.5 ml of RPM 1640 in a centrifuge tube. The tubes were then centrifuged at 1000 rpm for 10 min and cell pellets were washed once with 0.5 ml of PBS (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4, pH 7.3) and then fixed in 0.5 ml of methanol: glacial acetic acid (3.1) kept till examinations at +4 ^oC (27).

Smear preparation and staining of bone marrow cells were carried out according to Clarck (28) with modifications. Cell smears were prepared on clean glass slides, air-dried for 20-30 min and then stained with May-Grunwald followed by Giemsa stain. After washing in tap-water and then air-drying, slides were mounted with Entellan and screened under a light microscope (Olympus BX50) at 1000X magnification in oil for micronuclei formation of poly- and normochromatic erythrocytes (P and N cells respectively). Approximately 1500-2000

erythrocytes per animal were evaluated; the number of normochromatic and polychromatic erythrocytes were also scored and recorded. In addition, the ratio of P and N cells were also determined as a measure of bone marrow toxicity. The criteria for scoring and identification of micronuclei were similar to previous studies (10, 26).

The results were evaluated for significance using Student's t-test and the calculations were made using a computer program (SPSS).

Results

Mutagenicity of 2, 4, 5 triphenyl imidazole (Fig. 2) was investigated by scoring MN formation in Wistar rat bone marrow erythrocytes. Increasing doses of the compound were administered once with an interval of 24 h for 7 days, (Table 1). DMSO was also injected as control to a separate group of rats. Micronuclei formation was scored in P and N cells of bone marrow in treated and untreated (control) rats.

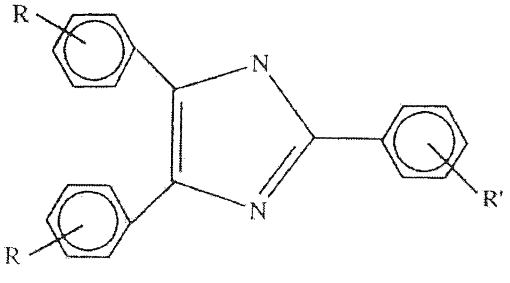

Fig. 1. The structural formulae of 2, 4, 5 triphenyl imidazole.

The results showed that 2, 4, 5 triphenyl imidazole produced a statistically significant increase in the percentage of MN ($p<0.05$ and $p<0.01$). The mutagenic activities are presented graphically as dose-response curves in Fig.2. As depicted, the percentages of micronuclei scored in total cells were very low (0.18 %) in untreated rats (negative controls) in comparison to other laboratory reports (10, 29). However it showed a dose-related increase from 2,4,5 triphenyl imidazole. Percentage of micronuclei in the bone marrow cells of 2-AAF treated rats (as positive controls) was found high as 4.1% of total erythrocytes. There was also a significant difference in the number of MN between DMSO and 2, 4, 5 triphenyl imidazole treated cells, as 2-3 times more in compound treated cells than the solvent controls. A dose dependent micronucleus formation was found 2-3 times more in N cells than that in P cells, as about 3% and 1-2.3%, respectively.

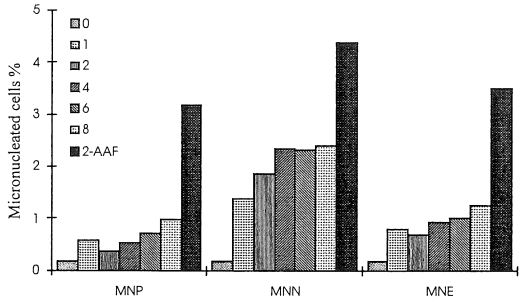

Fig.2. Percentage of micronucleated erythrocytes in bone marrow of rats after treatment with 2, 4, 5 triphenyl imidazole for 7 days. MNP, micronucleated P cells; MNN, micronucleated N cells; MNE, micronucleated erythrocytes. 0, (-controls); 1-8 mg/kg (doses of 2, 4, 5 triphenyl imidazole) 2-AAF, 150 mg/kg (+controls).

P and N cells were also scored for the P:N ratio in order to indicate the cytotoxicity of the test compound (14). The ratio between P and N cells did not reveal any significant toxic effect of the 2,4,5 triphenyl imidazole ($p>0.05$) on the erythropoiesis (Table 1). These results indicate that the compound causes chromosomal damage in the rat, but this is not very effective on the process of erythropoiesis.

Table 1. Induction of micronuclei in rat bone marrow following treatment with various concentrations of 2, 4, 5 triphenyl imidazole for 7 days.

Group	Dose (mg/kg)	P cells	N cells	MNP cells	B_{MNP} (%) within P	MNN cells	a_{MNN} (%) within N	$B_{MNP+MNN}$ (%)	P : N
Imidazol	1	1362	508	13	0.82	10	1.90	1.11	2.7
		1415	490	10		9			
	2	1427	423	11	0.86	9	2.1	1.23	3.4
		1453	407	14		12			
	4	1480	397	15	1.10	13	3.2	1.50	3.6
		1500	415	18		11			
	6	1416	467	17	1.33	17	3.6	1.88	3.1
		1350	422	20		15			
	8	1429	429	30	2.33	16	3.7	2.89	3.3
		1440	437	37		25			
	0	1315	563	2	0.18	0	0.0	0.18	2.5
		1372	511	3		2			
DMSO (ul/kg)	28	1432	453	4	0.24	2	0.4	0.31	3.0
		1430	500	3		3			
	57	1295	603	5	0.49	3	0.4	0.55	2.1
		1322	589	8		5			
	114	1410	492	7	0.57	2	0.4	0.57	2.8
		1390	505	9		4			
	172	1302	512	7	0.62	5	0.9	0.88	2.6
		1400	498	10		8			
	230	1323	519	16	1.34	10	1.9	1.63	2.5
		1350	528	20		15			
2-AAF (mg/kg)	150	1335	665	61	3.75	28	4.99	4.11	2.0
		1490	516	45		31			

Each dose treatment was based on 2 animals. DMSO, dimethyl sulphoxide (solvent control); 2-AAF, 2-acetamidofluorene (+ control); P, polychromatic cells; N, normochromatic cells; MNP, micronucleated P cells; MNN, micronucleated N cells; MNE, micronucleated erythrocytes. P:N, polychromatic normochromatic erythrocyte ratio. Statistically significant (Student's t-test) at: a- $p<0.05$, b- $p<0.01$.

Discussion

One of the important factors emerging from recent years is to evaluate consistent and specific cytogenetic studies for drugs, environmental pollutants and devices used for human health care system. The micronucleus test is a widely used experimental method as a part of the basic battery of assays to investigate the clastogenic effects of potential drugs (14, 30, 31). Micronuclei originated from the chromatin material are considerably smaller than the main nuclei. Formation of micronucleated cells in large numbers may be an indicator of the malignancy since 98% of human and animal tumors have structural and numerical chromosomal aberrations (32). Therefore, this test can predict the induction of structural aberrations caused by clastogenic potential (33, 34).

Imidazole is a simple heterocyclic molecule and forms different organic compounds. Its pKa value is around 7, allowing it to function as both proton donor and acceptor at physiological pH levels (18). There are several reports showing nonmutagenic and noncarcinogenic properties of imidazole and its derivatives (22, 23), whereas some derivates and imidazoles containing cobalt and cupper exhibit mutagenic effects (24, 34). However there is no evidence about the mutagenicity of 2, 4, 5 triphenyl imidazole.

In the present study, effects of 2, 4, 5 triphenyl imidazole on the formation of micronuclei in bone marrow erythrocytes of Wistar rats were investigated. To avoid any subjective error in cellular differentiation about 2000 erythrocytes (polychromatic and normochromatic) were scored from each animal. Ratio of MN in control group animals was found around 0.1-0.4 %, similar to the results obtained by other investigators (10, 35). A dose-dependent increase in the number of micronuclei was found, indicating the clastogenic effects of the compounds (Table 1). However, it is not toxic on erythropoeises process since the P:N ratios did not show significant increases. 2, 4, 5 triphenyl imidazole has a higher molecular weight than imidazole and its derivatives, such as hydantoin, hydantoic acid, N-acetyl-imidazole and histamin, therefore the size and the structure of the molecule may affect DNA or formation of spindle in mitosis as reported for some other derivatives of imidazole (24, 33, 36).

The test substance was also found to be more effective on N cells than P cells. This may be dependent on the development of N cells during erythropoesis since the formation of N cells follows P cells. Thus, long time exposure of N cells to the substance may cause high level damage on chromosomes or spindle formation during mitosis.

In conclusion, our observations suggest the *in vivo* susceptibility of mammals to the clastogenic, but not toxicity potential of 2,4,5 triphenyl imidazole. Our results do not represent a complete work of 2,4,5 triphenyl imidazole. We did not perform a comprehensive study of different cytotoxic end-points and no assay for the induction of chromosomal abnormalities has been included in this work yet, 2, 4, 5 triphenyl imidazole gave positive results in the assays performed and presented a reassuring safety profile.

References

1. Morita, T., Asano, N., Awogi, T., et al.: Mutat. Res.-Gen. Tox. En. Mutagen. 391(3) 259 (1997)
2. Schmid, W.: Mutat. Res. 31, 9 (1975)
3. Yamamoto, K.I. Kikuchi, Y.: I bid.71, 127 (1980)
4. Vanparys, P., Vermeiren, F., Sysmans, M. and Temmerman, R.: I bid. 244, 95 (1990)
5. Miller, B., Albertini, S., Locher, F., et al.: Mutat. Res.-Gen. Tox. En. Mutagen. 392 (1-2) 45 (1997)
6. Lu, P.J., Ho, I.C., Lee, T.C.: I bid. 414 (1-3) 15 (1998)
7. Mengs, U., Grimminger, W., Krumbiegel, G., Schuler, D., Silber, W., Volkner, W.: I bid. 444 (2) 421 (1999)
8. Adler, I.-D.: In Venitt,S., Parry, J.M. (Eds.) Mutagenicity Testing. A Practical Approach, pp. 275-306 IRL Press, Oxford 1984
9. Emecen, G., Ünlü, H.: Tr. J. Biology 19, 1 (1995)
10. Henderson, L., Fadyk, J., Windbank, S., Smith, M.: Mutat. Res. 291, 79 (1993)
11. Suzuki, T., Hayashi, A., Hakura, A.O., et al: Mutagenesis 10 (1) 31 (1995)

12. Kersten, B., Zhang, J., Brendler-Schwaab, S.Y., Kasper, P., Muller, L.: Mutat. Res.-Gen. Tox. En. Mutagen. 445 (1) 55 (1999)
13. Müller, W.U., Nüsse, M., Miller, B.M., et al: Mutat. Res. 366, 163 (1996)
14. Aaron, C.S., Sorg, R., Zimmer, D.: I bid. 223, 129 (1989)
15. Guadano, A., Gonzalez-Coloma, A., de la Pena, E.: Mutat. Res.-Gen. Tox. En. Mutagen. 414 (1-3) 1 (1998)
16. Mersch, J., Beauvais, M.N., Nagel, P.: Mutat. Res. 371, 47 (1996)
17. Ruiz, E.F., Rabopo, V.M., Zecona, S.V., et al: I bid. 270 (1) 45 (1992)
18. Nishie, K., Waiss, A.C., Keyl, A.C.: Toxicol. Appl. Pharmacol. 14, 301 (1969)
19. Işıkdağ, İ., Uçucu, Ü., Demirayak, Ş.: Tr. J. Med. Pharmacy 11 (2) 249 (1987)
20. Lombardino, J.G.: Pharmaceutical Imidazoles, Ger. Often. 2.155.558 (Cl. C 07 d) Ref: C.A. 77, 101607 y (1972)
21. Lombardino, J.G.: J. Med. Chem. 17(11) 1182 (1974)
22. Forster, R., Blowers, S.D., Cinelli, S., et al: Mutat. Res. 298, 71 (1992)
23. Voogd, C.E., van der Stel, J.J., Jacops, J.J.J.A.A.: I bid. 66, 207 (1979)
24. De, M.M., Vanelle, P., Bernadini, E., et al: Environ. Mol. Mutagen. 19 (2) 167 (1992)
25. Işıkdağ, İ., Uçucu, Ü., Çakır, B.: Gazi University J. Pharmacy 6 (1) 49 (1989)
26. Hayashi, M., Tice, R.R., MacGregor, J.T., et al: Mutat. Res. 312, 293 (1994)
27. Rooney, U.E., Czepulkowski, B.H.: Human Cytogenetics, A practical Approach, Oxford University Press, New York 1992
28. Clarck, G., Staining Procedures, William and Wilkins Press, Baltimore 1981
29. Krishna, G. (Ed.) Kropko, M.L., Ciaravino, V., Theiss, J.C.: Mutat. Res. 264, 29 (1991)
30. OECD Guideline For Testing Of Chemicals-474. Genetic Toxicology; Micronucleus Test. The OECD Catalogue Pub. Paris Cedex 16 pp.1-6, 1984
31. International Standardisation Organisation (ISO), Biological Evaluation Of Medical Devices. Tests for Genotoxicity, Carcinogenecity and Reproductive Toxicity 10993-3 Switzerland pp. 1-5, 1992
32. Sobti, R.C., Bhardwaj, D.K., Gupta, B.D.: Med. Sci. Res. 19, 23 (1991)
33. Heddle, L.A., Hite, M., Kirkhart, B., et al: Mutat. Res.123, 61 (1983)
34. Schmid, W.: Agents Actions 3/2, 77 (1973)
35. Hartman, Z., Hartman, P.E.: Chem. Biol. Interact. 84 (2) 153 (1992)
36. Basu, A.K., Wood, M.L., Niedernhofer, L.J., et al: Biochemistry 32 (47) 12793 (1993)

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