Mitoclastic and Clastogenic Effects of Aspirin on Mouse Bone-marrow Cells *in vivo*

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Aspirin (acetyl salicylic acid) originally extracted from the willow (*Salix alba*) bark is a widely used antipyretic, analgesic, antirheumatic and antithrombotic drug freely available without medical prescription. Recently, it has been recommended for the primary prevention of myocardial infarction in patients with stable angina pectoris (Juul-Moller *et al.* 1992). However, several adverse side effects including its interference in platelet function have been known for a long time (Griffin and D'Arcy 1979). Gilman *et al.* (1991) have also warned of its adverse effects, if not administered properly. Weissmann (1991) has presented an excellent review on the history, uses and adverse side effects of aspirin. Odashima (1976) was the first to show its carcinogenic potentiality from animal experiments, especially transplacental carcinogenesis. Epidemiological studies in human beings have indicated that when taken together with phenacetin, aspirin increases the risk of urothelial cancer, particularly of the renal pelvis (IARC 1987, McCredie *et al.* 1993). Experimental studies of the U.S. Environmental Protection Agency have also confirmed the carcinogenic potential of aspirin (Nesnow *et al.* 1987). On the other hand, some reports show that aspirin intake reduces colon cancer incidence in man (Kune *et al.* 1988, Thun *et al.* 1991) and that has been explained to be due to the inhibition of arachidonic acid metabolism by aspirin (Marnett 1992). Hence, it appeared reasonable to test this drug in mouse bone marrow system to find out whether it has any mitoclastic or clastogenic properties.

Materials and methods

Aspirin in the form of water soluble tablets with the trade name "Dispirin" manufactured by Rekitt and Colman of India Ltd. (batch Nos. M-2272 dated 8.1.1991 and M-2668 dated 1/1992) was used in the present experiments. Three different concentrations of aspirin viz., 100 µg/ml, 10 µg/ml and 1 µg/ml, were prepared in glass distilled water and sterilised by millipore filtration. The test animals used were inbred Swiss albino mice (*Mus musculus* 2n=40) with a body weight around 30 g and aged three months. Four mice were taken in each batch and each one was given 1 ml of 100 µg/ml, 10 µg/ml and 1 µg/ml of aspirin solution, respectively and the fourth one kept as control was given 1 ml distilled water, all by intraperitoneal (I.P.) injection. Although for i.p. injections, sterile physiological saline is used as the solvent of several drugs, it was avoided in this experiment as there is the possibility of the formation of sodium salicylate by chemical interaction of aspirin with saline (sodium chloride). After 24 hr, every one of the mice was sacrificed by quick cervical dislocation and the femurs were excised. Both ends of the femur were cut open with scissors and the fixative (methyl alcohol and acetic acid in 3:1 ratio) was flushed through the narrow cut end of the bone, using a hypodermic syringe to eject the bone marrow intact and was preserved in the fixative duly labelled. On the next day, Leishman smears and Acetocarmine squashes were prepared from the bone marrow samples and the slides

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were examined under the microscope. The experiments were conducted in triplicate.

Observations

Bone marrow pictures of mice kept as control were normal, whereas the marrow from aspirin treated mice showed a wide range of cytological abnormalities. A marked lowering of the mitotic index with increasing concentration of the drug was noted (Table 1).

Fig. 1 shows a general picture of the bone marrow from a mouse treated with 1 μ g/ml aspirin. A few cells arrested at prophase and one arrested at anaphase with sticky bridges can be seen. Figs. 2 and 3 represent mitotic arrest at prophase along with stickiness of chromosomes. Such mitotic arrests after chromosome condensation led to the formation of pycnotic nuclei (Figs. 4, 5). In Fig. 5, two other nuclei with prominent central vacuoles can also be seen. Blebbing of pycnotic nuclei (Fig. 6) was another abnormality encountered. Micronuclei (Fig. 7) were seen in all treated marrows. Fig. 8 shows two adjacent cells in prophase, with some degree of chromosome stickiness, of which one appears to be polyploid. In Fig. 10, three adjacent cells are in prophase of which two are evidently polyploid. In these also, some degree of stickiness is there. Abnormal metaphase configurations such as clumped metaphase and hollow metaphase (Fig. 9) were often encountered. Pulverization of chromosomes resulting in the formation of chromatin globules of varying sizes as in Fig. 11 was observed in bone marrow cells of mice administered with 10 μ g/ml aspirin. Mitotic arrest at anaphase was also noticed (Fig. 12). Anaphases with sticky bridges (Fig. 13) were also encountered in treated bone marrow and such sticky bridges eventually disappear (Fig. 14). Pycnotic telophase nuclei with projecting telomeres were also observed occasionally (Fig. 15). The various cytological abnormalities induced by aspirin at the three different concentrations tested in mouse bone marrow *in vivo* are summarised in Table 2 along with controls.

Discussion

The above observations clearly show that aspirin is both mitoclastic and clastogenic even at the lowest concentration used. The mitoclastic property of the drug is evidenced from the

Table 1. Mitodepressive effect of aspirin on the bone marrow cells of inbred Swiss albino mice

Concentration of aspirin	Duration of treatment	Number of mice used	Total number of bone marrow cells examined	Number of dividing cells	Mitotic index
0 (Distilled water)	24 hr.	3	3451	2539	73.57
			3434	2546	74.14
			3436	2541	73.95
			3440 $\pm$ 5.36	2542 $\pm$ 2.08	73.84 $\pm$ 0.17
Mean					
1 μ g/ml	24 hr.	3	3483	1829	52.51
			3477	1838	52.86
			3492	1830	52.41
			3484 $\pm$ 4.36	1832 $\pm$ 2.85	52.59 $\pm$ 0.14
Mean					
10 μ g/ml	24 hr.	3	3522	674	19.14
			3495	688	19.69
			3525	700	19.86
			3514 $\pm$ 9.54	687.33 $\pm$ 7.51	19.56 $\pm$ 0.22
Mean					
100 μ g/ml	24 hr.	3	3417	301	8.81
			3404	289	8.49
			3415	297	8.70
			3412 $\pm$ 4.04	295.7 $\pm$ 3.53	8.667 $\pm$ 0.09
Mean

Figs. 1–15. Cytological abnormalities induced in bone marrow cells of inbred Swiss mice given IP injection and with different concentrations ($1\text{ }\mu\text{g/ml}$, $10\text{ }\mu\text{g/ml}$, $100\text{ }\mu\text{g/ml}$) of aspirin (All figures $\times 1150$). (1) General view of the bone marrow ($1\text{ }\mu\text{g/ml}$ aspirin), (2) Stickiness and restitutions ($1\text{ }\mu\text{g/ml}$), (3) Restitution at prophase ($10\text{ }\mu\text{g/ml}$), (4) Nuclear pycnosis ($100\text{ }\mu\text{g/ml}$), (5) 2 Pycnotic nuclei ($100\text{ }\mu\text{g/ml}$), (6) Nuclear blebbing ($100\text{ }\mu\text{g/ml}$), (7) Budding of 2 micronuclei ($100\text{ }\mu\text{g/ml}$), (8) Prophase chromosomes from 2 adjacent cells exhibiting stickiness ($100\text{ }\mu\text{g/ml}$), (9) Hollow metaphase ($1\text{ }\mu\text{g/ml}$), (10) Polyploidy and Stickiness ($10\text{ }\mu\text{g/ml}$), (11) Pulverization of metaphase chromosomes resulting in the formation of chromatin globules ($10\text{ }\mu\text{g/ml}$), (12) Late Anaphase with a sticky bridge ($100\text{ }\mu\text{g/ml}$), (13) Anaphase ($10\text{ }\mu\text{g/ml}$) with multiple sticky bridges, (14) A pair of persistent bridges between 2 telophase nuclei ($10\text{ }\mu\text{g/ml}$), (15) Broken bridge between two pycnotic telophase nuclei ($100\text{ }\mu\text{g/ml}$).

lowering of the mitotic index as well as from the arrest of the M-Phase substages and from the manifestation of spindle abnormalities. Of these, the lowering of the mitotic index might have been achieved by the inhibition of DNA synthesis at S-phase or by the arrest of cell cycle at G_2 -phase. Greater the concentration of the drug, the lower was the mitotic index. In this respect, aspirin was found to be stronger than paracetamol, analgin, palgin and phenylbutazone of equivalent concentration tested in this laboratory (Stephen *et al.* 1994). Mitotic arrest in all the sub-stages of M-phase suggests that aspirin action is not stage-specific. According to Nagl (1970), blockage of the cell cycle at G_2 -phase results in endoreduplication, blockage at prophase

Table 2. Cytological abnormalities induced by aspirin in the normoblasts from the bone marrow of inbred Swiss albino mice

Conc. of aspirin	Duration of treatment	Number of mice	Total number of cells examined	Percentage of normoblasts	Percentage of normal normoblasts	Percentage of normoblasts with								Total
						Nuclear Pycnosis	Micro-nuclei	Clumping of chromo-somes	Poly-ploidy	Hollow metaphase	Bridges at anaphase	Restitution	Other abnormalities	
0 (Distilled water)	24 hr.	3	3942	5.26	5.26	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
			3964	5.28	5.28	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
			3965	5.27	5.27	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mean			3957	5.27	5.27									
			± 7.51	± 0.01	± 0.01									
1 µg/ml	24 hr.	3	3894	4.98	3.71	0.29	0.43	0.21	0.05	0.04	0.20	0.02	0.03	1.27
			3918	5.02	3.67	0.32	0.44	0.21	0.07	0.03	0.22	0.02	0.04	1.35
			3927	5.03	3.72	0.29	0.39	0.24	0.06	0.05	0.21	0.02	0.05	1.31
Mean			3913	5.02	3.70	0.30	0.42	0.22	0.06	0.04	0.21	0.02	0.04	1.31
			± 9.85	± 0.02	± 0.02	± 0.01	± 0.02	± 0.01	± 0.01	± 0.01	± 0.01	± 0.00	± 0.01	± 0.02
10 µg/ml	24 hr.	3	4009	4.88	1.91	0.93	0.86	0.36	0.20	0.01	0.44	Nil	0.09	2.89
			3986	4.93	1.94	0.92	0.86	0.38	0.22	0.01	0.41	Nil	0.13	2.93
			3996	4.95	1.94	0.91	0.84	0.37	0.21	0.01	0.44	Nil	0.11	2.89
Mean			3997	4.92	1.93	0.92	0.85	0.37	0.21	0.01	0.43	Nil	0.11	2.90
			± 6.66	± 0.02	± 0.01	± 0.01	± 0.01	± 1.01	± 0.01	± 0.00	± 0.01		± 0.01	± 0.01
100 µg/ml	24 hr.	3	3942	4.98	0.55	2.48	1.0	0.24	0.01	Nil	0.49	Nil	0.13	4.35
			3957	4.96	0.59	2.46	1.2	0.27	0.01	Nil	0.54	Nil	0.12	4.60
			3951	4.97	0.58	2.47	1.1	0.27	0.01	Nil	0.53	Nil	0.14	4.52
Mean			3950	4.97	0.57	2.47	1.1	0.26	0.01	Nil	0.52	Nil	0.13	4.49
			± 4.36	± 0.01	± 0.01	± 0.01	± 0.06	± 0.01	± 0.00		± 0.02		± 0.01	± 0.07

before nuclear membrane dissolution results in endomitosis and blockage at metaphase results in C-mitosis.

As the polyploid numbers of condensed chromosomes presently observed were not limited by the nuclear membrane, these would have been formed by mitotic arrest at metaphase as caused by colchicine and vinca alkaloids (Bieseke 1958, Deysson 1968). Acute arrests at prometaphase and metaphase would have resulted in chromosome clumping, hollow metaphase and highly pycnotic nuclei. Moreover, the drug was capable of arresting mitosis at anaphase and early telophase stages resulting in the formation of irregular pycnotic daughter nuclei. These abnormalities were also noticed in treatment with another antipyretic and analgesic drug, analgin (Meenakumari and Stephen 1995). In fact, many of the mitotic poisons are capable of causing these abnormalities (Bieseke 1958, Deysson 1968). Shanthamurthy and Rangaswamy (1979) had reported mitotic spindle abnormalities induced by aspirin in *Allium cepa*.

Regarding the clastogenic potentiality, aspirin is capable of causing chromosome fragmentation and the formation of micronuclei. Bone marrow micronucleus test (Schmid 1975, Maier and Schmid 1976) has been approved as a reliable test for the clastogenicity of chemicals. Mouse bone marrow chromosome assay is also a standard short-term clastogenicity assay (Larramendy *et al.* 1980). Besides these, chromosome stickiness and sticky bridges were encountered in treated materials. According to Klasterska *et al.* (1976), sticky bridges at anaphase represent subchromatid aberrations. Many of these chromosomal aberrations reported to be induced by other clastogenic chemicals (Kihlman 1966). The acute pulverization of almost all the chromosomes in the bone marrow cells resulting in the formation of numerous chromatin globules as presently observed has been reported in *A. cepa* cells treated with the antineoplastic drug Adriamycin (Mercykutty and Stephen 1980) and points out to the possibility of the destruction of bone marrow cells upon aspirin administration and hence bone marrow depression. Griffin and D'Arcy (1979) had mentioned about the deleterious effect of aspirin on platelet function. Gilman *et al.* (1991) have commented: "Despite the efficacy and safety of aspirin as an analgesic and antirheumatic agent, it is necessary to be aware of its role in Reye's syndrome and a common cause of lethal drug poisoning in children, as well as its potential for serious toxicity, if used improperly." Even though the drug may be highly useful in the prevention or management of cardio-vascular diseases (Juul-Moller *et al.* 1992), the presently observed highly mitoclastic and clastogenic effects induced in mouse bone marrow cells, coupled with the reports of carcinogenic properties exhibited by this drug (Odashima 1976, Nesnow *et al.* 1987, IARC 1987) demand for the abolition of indiscriminate use of this drug without medical prescription.

Summary

One ml each of sterile aqueous solutions of aspirin (acetyl salicylic acid) at 1 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$ were injected intraperitoneally into different inbred Swiss albino mice of about 30 g weight. After 24 hr, bone marrow was extracted from the femurs and cytological preparations were made. There was a marked depression in the mitotic indices of the bone marrow cells, in treated mice corresponding to the concentration of aspirin compared to that of controls. Mitotic arrests and spindle abnormalities were also encountered. The drug was also capable of inducing chromosome fragmentation, pulverization, stickiness and sticky bridges. These observations clearly show that aspirin is both mitoclastic and clastogenic even at very low concentrations, and hence its indiscriminate use has to be discouraged.

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