International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **7(3),** 36-40, March **(2018)** *Int. Res. J. Biological Sci.*

Testing of genotoxic potential of amikacin sulphate through micronucleus test in a fish in vivo system

Manas Ranjan Dash[1*] and Dhananjaya Soren[2]
[1]Department of Zoology, B.B. Mahavidyalaya, Harichandanpur, Keonjhar-758028,Keonjhar Dist., Odisha, India
[2]Department of Zoology, Ravenshaw University, Cuttack-753003, Cuttack Dist., Odisha, India
197manas@gmail.com

Available online at: www.isca.in, www.isca.me
Received 4th January 2018, revised 2nd March 2018, accepted 9th March 2018

Abstract

In the present study, the injectable form of Amikacin (Amikacin sulphate) a therapeutically popular broad spectrum antibiotic, is assessed for its genotoxic potential using MNT in a fish, Oreochromis mossambicus. *Amikacin sulphate is already reported to be potentially nephrotoxic, ototoxic and neurotoxic. In the present investigation, Amikacin sulphate failed to induce MN in lower doses but it did so in higher doses, though not significantly. Again, the frequencies of MN and nuclear abnormalities (NA) were found to be comparatively higher in fishes exposed to the antibiotic for longer period irrespective of strength of the treatment dose. In comparison to MN frequencies, the frequencies of nuclear abnormalities (NA) were observed to be higher in fishes of all treated groups and their incidences were dose and exposure dependent. The frequencies of both MN and nuclear abnormalities showed an increasing trend with the increase of dose strength and exposure time. The MN and NA data of this study indicated that Amikacin sulphate has the potentiality to cause damage or instability to the genome of an organism in higher doses and longer exposures. However, long term studies in animals employing different assay systems are required to assess its carcinogenic and genotoxic potential thoroughly.*

Keywords: Testing, genotoxic, potential, amikacin, sulphate, micronucleus, fish, vivo system.

Introduction

Human beings today are exposed to innumerable synthetic chemicals in their day-to-day life in some form or the other. Many of these chemicals have been proved to have toxic potential to cause health hazards not only in humans but also in other organisms in our surroundings. One of the most important group of synthetic chemicals is the pharmaceutical compounds, a significant portion of which is the antibiotics. Despite their therapeutic importance, many antibiotics are found to be clastrogenic, teratogenic, neurotoxic, ototoxic, genotoxic or carcinogenic as assessed through different assay systems[1-7].

A good number of leftover pharmaceutical compounds including antibiotics find their way to aquatic ecosystems causing serious problems at various levels of the life of aquatic organisms as well as human beings. Amikacin, a broad spectrum antibiotic, is a semisynthetic derivative of a aminoglycoside antibiotic, kanamycin. Despite its many therapeutic and other advantages, Amikacin is reported to cause nephrotoxicity, ototoxicity and to certain extent genotoxicity[8]. Genotoxic potential of Amikacin has not been tested extensively in different test organisms using different assay systems.

Among the currently available assay systems, the micronucleus test (MNT) is the most widely employed test protocol for its simplicity, reliability and sensitivity. This test was first used in a fish system in laboratory[9] and since then MNT test in fish erythrocytes from peripheral blood is being used for assessing genotoxicity in aquatic ecosystems and genotoxic potential of different chemicals in the laboratory[10-15]. Through genotoxic testing of Amikacin employing MNT in this study, we intend to add some more information on the safety evaluation of this broad spectrum antibiotic for its extensive human use.

Materials and methods

The test animal of this study was *Oreochromis mossambicus*, a widely available local fish. The specimens were procured from the local market or from local water bodies of Keonjhar District of Odisha, India. The collected fish were acclimatized to the laboratory conditions in aquaria for about a week preceding treatment for experiments.

The test chemical was Amikacin sulphate procured from a drug store. It was in the form of injectable solution containing 100 mg Amikacin sulphate in 2 ml solvent (water) produced and marketed by Aristo Laboratories Pvt. Ltd., Makhnumajra, Solan District, Himachal Pradesh, India under a brand name MIKACIN.

Sublethal stock solutions were prepared from the injectable solution. In this study, the chosen experimental doses were in the measure of mg/kg body weight and the rate of administration was 1 ml/100 gm body weight. The experimental doses of the test chemical of this study were 50mg, 100mg,

200mg, 300mg and 400mg per kg body weight. These sublethal doses were prepared and injected to the fish specimens on the basis of the toxicological data already available and keeping in view the recommended doses of the drug for humans. The specimens of the control group were injected with distilled water (the solvent of the test drug) at the rate of 1 ml. per kg body weight.

The experimental fish were injected IM at every 24hour duration i.e., twice for 24hour group and thrice for 48 hr. group. After 6 hours of administration of the last dose, a small incision was made near the tail to draw blood for smear preparation. Slides were air dried, fixed is absolute methanol and stained with Leishman's-Giemsa's stains. The stained slides were dried in the air and observed under oil immersion objective of a binocular light microscope. About 1000 erythrocytes were scored from the slides of fish treated with a particular concentration of the test chemical for detection of MN and other nuclear abnormalities. Frequencies of cells with MN and those with other nuclear abnormalities were subjected to statistical analyses like S.D. and ANOVA calculations to test their significance.

Results and discussion

The scored micronuclei and nuclear abnormalities, and their percentages are presented in Tables-2-4. The normal erythrocyte of the test fish, *Oreochromis mossambicus* has a elliptical nucleus. On treatment with some of the doses of the test chemical, there was induction of micronuclei which were small, non-refractile and circular or ovoid particles, isochromatic with the main nucleus lying in the cytoplasm. These micronuclei were found to vary in size but the number was never more than one per cell. The location of the micronucleus in the cytoplasm was also found to be variable. In addition to micronucleus formation, almost all doses of the test chemical (Amikacin sulphate) also induced abnormalities in the shape of nuclei. These nuclear abnormalities were classified as blebbed (BL), lobed (LB), notched (NT) and binucleated cells (BN). An erythrocyte with two nuclei of equal size and identical stain found within the same cytoplasmic boundary was designated as binucleated. If there was a small evagination of nuclear membrane containing euchromatin, it was considered as blebbed and if the evagination was large enough to produce several lobes in the nucleus, it was considered as lobed. A deep concavity in the nucleus was classified as notched nucleus.

There was no induction of micronucleus in any of the 1045 scored erythrocytes of the control group injected with glass double distilled water. Similarly no micronuclei were observed in any of the smears of the fish of the 24 hours experimental group injected with any of the 50 mg, 100 mg and 200 mg doses of the test chemical. However, different kinds of other nuclear abnormalities were observed in the erythrocytes in these treatment groups of fish (control, 50 mg, 100 mg and 200 mg). Such nuclear abnormalities showed a dose dependent increase from control to 200 mg dose (total NAs being 13, 18, 24 and 24 respectively). Only one erythrocyte was observed to possess a MN from amongst 1013 erythrocytes scored from 300 mg treated fish of 24 hour group. Out of 1002 scored erythrocytes of 400 mg experimental group, three cells exhibited MN. In these two latter groups (300 mg and 400 mg), there was a steady increase in the number of nuclear abnormalities (the number of NA being 32 and 41 respectively).

Table-1: Frequencies of micronuclei and nuclear abnormalities induced by different doses of Amikacin sulphate in erythrocytes of *Oreochromis mossambicus* after 24 hours of treatment.

Treatment doses in mg/kg	No. of specimens used	No. of erythrocytes scored	Number of types of mutagenic effects					
			Micronuclei (MN)	Nuclear abnormalities (NA)				
				Blebbed (BL)	Lobed (LB)	Notched (NT)	Binucleated (BN)	Total
Control	8	1045	0	3	5	2	3	13
50	8	1065	0	3	7	5	3	18
100	8	1020	0	2	7	8	7	24
200	8	1028	0	5	10	3	6	24
300	8	1013	1	4	13	5	10	32
400	8	1002	3	11	17	5	8	41

Table-2: Percentage frequencies of micronuclei and nuclear abnormalities induced by different doses of Amikacin sulphate after 24 hours of Treatment.

Treatment doses in mg/kg	No. of specimens used	No. of erythrocytes scored	Frequency of types of mutagenic effects in %					
			Micronuclei (MN)	Nuclear abnormalities (NA)				
				Blebbed (BL)	Lobed (LB)	Notched (NT)	Binucleated (BN)	Total
Control	8	1045	0	0.287+ 0.126	0.478+ 0.436	0.287+ 0.416	0.287+ 0.392	1.244+ 0.012
50	8	1065	0	0.281+ 0.003	0.657+ 0.423	0.469+ 0.108	0.281+ 0.146	1.690+ 0.313
100	8	1020	0	0.197+ 0.319	0.686+ 0.002	0.784+ 0.654	0.686+ 0.263	2.352+ 0.421
200	8	1028	0	0.486+ 0.006	0.972+ 0.001	0.291+ 0.035	0.583+ 0.121	2.334+ 0.002
300	8	1013	0.098+ 0.321	0.394+ 0.002	1.283+ 0.043	0.493+ 0.169	0.987+ 0.016	3.158+ 0.193
400	8	1002	0.299+ 0.161	0.097+ 0.112	1.696+ 0.663	0.499+ 0.813	0.798+ 0.216	4.091+ 0.692

Table-3: Frequencies of micronucleus and nuclear abnormalities induced by different doses of Amikacin Sulphate in erythrocytes of *Oreochromis mossambicus* after 48 hours of treatment.

Treatment doses in mg/kg	No. of specimens used	No. of erythrocytes scored	Number of types of mutagenic effects					
			Micronuclei (MN)	Nuclear abnormalities (NA)				
				Blebbed (BL)	Lobed (LB)	Notched (NT)	Binucleated (BN)	Total
Control	8	1061	0	2	4	3	5	14
50	8	1072	0	3	8	4	6	21
100	8	1009	1	3	6	10	9	28
200	8	1002	1	5	11	3	8	27
300	8	1020	3	6	12	7	11	39
400	8	1051	3	10	20	6	8	42

Table-4: Percentage frequencies of micronuclei and nuclear abnormalities induced by different doses of Amikacin sulphate after 48 hours of Treatment.

Treatment doses in mg/kg	No. of specimens used	No. of erythrocytes scored	Frequency of types of mutagenic effects in %					
			Micronuclei (MN)	Nuclear abnormalities (NA)				
				Blebbed (BL)	Lobed (LB)	Notched (NT)	Binucleated (BN)	Total
Control	8	1061	0.00+ 0.00	0.188+ 0.114	0.377+ 0.233	0.282+ 0.003	0.471+ 0.819	1.319+ 0.018
50	8	1072	0.00+ 0.00	0.279+ 0.020	0.746+ 0.002	0.373+ 0.008	0.559+ 0.034	1.958+ 0.311
100	8	1009	0.099+ 0.120	0.297+ 0.016	0.594+ 0.039	0.991+ 0.129	0.891+ 0.129	2.775+ 0.119
200	8	1002	0.099+ 0.211	0.499+ 0.132	1.097+ 0.337	0.299+ 0.032	0.798+ 0.062	2.694+ 0.066
300	8	1020	0.294+ 0.201	0.588+ 0.421	1.176+ 0.067	0.686+ 0.716	1.078+ 0.115	3.823+ 0.305
400	8	1051	0.285+ 0.612	0.951+ 0.032	1.902+ 0.981	0.570+ 0.004	0.761+ 0.016	3.996+ 0.098

In the 48 hours treatment group, MN induction was totally absent in the control as well as 50 mg treated fish. Only one erythrocyte with MN was observed in each of 100 mg and 200 mg treated group. In the smears of 300 mg and 400 treated fish, the micronuclei induced were 3 each. Thus there was a dose – dependent increase in the number of MN induced in the 48 hour exposure group. As to nuclear abnormalities, the same dose – dependent increase trend was also observed. The numbers of abnormalities were found to be 14, 21, 28, 27, 39 and 42 in the control, 50 mg, 100 mg, 200 mg, 300 mg and 400 mg treated fish respectively in this exposure group.

Despite the trend of increase in the number of micronuclei and nuclear abnormalities concomitant with the increase in the concentration of Amikacin sulphate and also with the increase of exposure time, the observed figures were either insignificant or on the border line of significance levels.

Discussion: The micronucleus test (MNT) is one of the widely employed test systems to assess the genotoxicity caused by a variety of synthetic chemicals and pollutants as it is simple, rapid and easy technique and targets mitotically active cell population in their interphase with any karyotype[14]. Low DNA content in cells, small chromosomes and low mitotic index make many species of fish unsuitable to study toxic effect of chemicals at chromosome level. Since MNT remains unaffected by the above parameters, it can conveniently be applied to fish to assess genotoxic potential of any chemical.

Amikacin is a therapeutically popular antibiotic and is extensively preferred as it is a rapid acting, cheap and effective antibiotics[8,16-18]. Despite several advantages, Amikacin is known to be ototoxic, nephrotoxic and neurotoxic depending on strength of dose[19-22]. There were reports of DNA damage in rats by Amikacin[8] and DNA and RNA damage by Cu (11) – amikacin complex[23]. Amikacin sulphate injection USP is generally prescribed for treating serious gram- negative bacterial infections for a short term and is reported to be potentially nephrotixic, ototoxic and neurotoxic. Long term studies in animals to evaluate carcinogenic potential of Amikacin sulphate has not been undertaken and mutagenicity of this drug has not yet been studied[24].

The present study clearly showed that lower doses of Amikacin sulphate fail to induce MN while the higher doses did induce but not significantly. Again induction of MN was found to rise with increase in exposure time. More micronuclei were observed in the smears of 48 hour exposure group irrespective of dose strength. Nuclear abnormalities were also increased in number concomitant with both increase in drug concentration and increase in exposure time. The MN data indicated that though Amikacin sulphate was capable of inducing genotoxicity in the erythrocytes of fish, *Oreochromis mossambicus*, it did so mildly. In comparison to MN frequency, the frequencies of nuclear abnormalities (NA) were observed to be higher in the fishes of all treated groups and their incidences were dose and exposure dependent. All categories of NA were observed in the erythrocytes of fish of each treatment group and of each exposure. The NA data of experimental groups appeared statistically significant when compared to those of the control group. Thus, the NA data of this study indicated that Amikacin sulphate has the potentiality to cause damage or instability to the genome of an organism in higher doses or longer exposures especially to that of aquatic animals like fish. It was demonstrated that Amikacin could induce damage to the DNA of lymphocytes in the blood of rats[8].

Another aminoglycoside antibiotic Gentamicin has shown negative results in a series of *in vivo* and *in vitro* genotixic studies[25]. However, positive results were noted in *in vitro* tests for forward mutation in *E.coli,* in a test for chromosomal aberration in mouse L-cells and in a test for sister chromatid exchange in human fibroblasts[25]. The conclusion of EMEA is that gentamicin is unlikely to be genotoxic. One more aminoglycoside antibiotic Neomycin sulphate was also reported to be genotoxically negative[26,27]. All these studies fail to prove conclusively that aminoglycoside antibiotics are potentially genotoxic.

Our study indicates that Amikacin sulphate is not a serious genotoxic antibiotic rather it can induce a certain degree of genotoxicity at higher doses and longer exposures. Long term studies in animals employing different assay systems are required to assess its carcinogenic and genotoxic potential thoroughly.

Conclusion

The present study reveals that Amikacin sulphate is moderately genotoxic especially in higher dosages and longer exposure

References

1. Mačor M. and Ebringer L. (1988). Effect of elevated temperature on genotoxicity of chemotherapeuticals towardEuglena gracilis. *Folia microbiologica*, 33(4), 314.

2. McQueen C.A., Way B.M., Queener S.M., Schlüter G. and Williams G.M. (1991). Study of potential in vitro and in vivo genotoxicity in hepatocytes of quinolone antibiotics. *Toxicology and applied pharmacology*, 111(2), 255-262.

3. Gibson D.P., Ma X., Switzer A.G., Murphy V.A. and Aardema M.J. (1998). Comparative genotoxicity of quinolone and quinolonyl-lactam antibacterials in the in vitro micronucleus assay in Chinese hamster ovary cells. *Environmental and molecular mutagenesis*, 31(4), 345-351.

4. Ila H.B. and Topaktas M. (1999). In vivo genotoxic effects of spiramycin in rat bone marrow cells. *Cytologia*, 64(3), 277-283.

5. Sontakke Y.A. and Fulzele R.R. (2009). Cytogenetic study on genotoxicity of antitumor-antibiotic Mitomycin C.

Biomedical Research, 20(1), 40-44.

6. Çelik A. and Eke D. (2011). The assessment of cytotoxicity and genotoxicity of tetracycline antibiotic in human blood lymphocytes using CBMN and SCE analysis, in vitro. *International Journal of Human Genetics*, 11(1), 23-29.

7. Metovic A., Mackic-Djurovic M. and Ibrulj S. (2013). Analysis of chromosome aberrations contained in vitro human peripheral blood lymphocytes after treatment with ceftriaxone. *Medical Archives*, 67(4), 228.

8. Koşar P.A., Aşcı H., Ciğerci I.H., Saygın M., Calapoğlu M., Yüksek Ş. and Cankara F.N. (2017). The Effect of Alpha-Lipoic Acid on Preventing Amikacin-Induced DNA Damage in Rats. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 87(4), 1489-1495.

9. Hooftman R.N. and De Raat W.K. (1982). Induction of nuclear anomalies (micronuclei) in the peripheral blood erythrocytes of the eastern mudminnow Umbra pygmaea by ethyl methanesulphonate. *Mutation Research Letters*, 104(1-3), 147-152.

10. Belpaeme K., Delbeke K., Zhu L. and Kirsch-Volders M. (1996). Cytogenetic studies of PCB77 on brown trout (Salmo trutta fario) using the micronucleus test and the alkaline comet assay. *Mutagenesis*, 11(5), 485-492.

11. Hayashi M., Ueda T., Uyeno K., Wada K., Kinae N., Saotome K., Tanaka N., Takai A., Sasaki Y.F., Asano N., Sofuni T. and Ojima Y. (1998). Development of genotoxicity assay systems that use aquatic organisms. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 399(2), 125-133.

12. Rodriguez-Cea A., Ayllon F. and Garcia-Vazquez E. (2003). Micronucleus test in freshwater fish species: an evaluation of its sensitivity for application in field surveys. *Ecotoxicology and Environmental Safety*, 56(3), 442-448.

13. Ali F., El-Shehawi A.M. and Seehy M.A. (2008). Micronucleus test in fish genome: a sensitive monitor for aquatic pollution. *African journal of biotechnology*, 7(5), 606-612.

14. Bolognosi C. and Hayashi M. (2011). Micronucleus assay in aquatic animals. *Mutagenesis*, 26(1), 205-213.

15. Bucker A., Carvalho M., Conceição M. and Alves-Gomes J. (2012). Micronucleus test and comet assay in erythrocytes of the Amazonian electric fish Apteronotus bonapartii exposed to benzene. *Ecotoxicology and Environmental Contamination*, 7(1), 65-73.

16. Kadurugamuwa J.L., Clarke A.J. and Beveridge T.J. (1993). Surface action of gentamicin on Pseudomonas aeruginosa. *Journal of bacteriology*, 175(18), 5798-5805.

17. Begg E.J. and Barclay M.L. (1995). Aminoglycosides – 50 years on. *Br. J. Clin. Pharmacol*., 39, 597-603.

18. Poulikakos P. and Falagas M.E. (2013). Aminoglycoside therapy in infectious diseases. *Expert opinion on pharmacotherapy*, 14(12), 1585-1597.

19. Szczepanik W., Kaczmarek P. and Jeżowska-Bojczuk M. (2004). Oxidative activity of copper (II) complexes with aminoglycoside antibiotics as implication to the toxicity of these drugs. *Bioinorganic chemistry and applications*, 2(1-2), 55-68.

20. Ozer M.K., Asci H., Oncu M., Yesilot S., Savran M., Bayram D. and Cicek E. (2009). Effects of pentoxifylline on amikacin-induced nephrotoxicity in rats. *Renal failure*, 31(2), 134-139.

21. Aksoy F., Dogan R., Ozturan O., Eren S.B., Veyseller B., Pektas A. and Hüseyinbas Ö. (2014). Protective effect of trimetazidine on amikacin-induced ototoxicity in rats. *International journal of pediatric otorhinolaryngology*, 78(4), 663-669.

22. Asci H., Saygin M., Cankara F.N., Bayram D., Yesilot S., Candan I.A. and Ilhan I. (2015). The impact of alpha-lipoic acid on amikacin-induced nephrotoxicity. *Renal failure*, 37(1), 117-121.

23. Jeżowska-Bojczuk M., Szczepanik W., Leśniak W., Ciesiołka J., Wrzesiński J. and Bal W. (2002). DNA and RNA damage by Cu (II)-amikacin complex. *The FEBS Journal*, 269(22), 5547-5556.

24. Drugs.Com (2017), Amikacin. https//www.drugs.com/pro/amikacin.html. Acessed on Oct 13, 2017.

25. EMEA (2017). The Europian Agency for Evaluation of Medicinal Products, Veterinary Medicines and Inspections, 2001. Committee for veterinary medicinal products, Gentamicin, Summary Report (3). Available at http://www.emea.eu.int., Accessed on Oct 13, 2017.

26. International programme on chemical safety website (2017). WHO food additives series 41 prepared by the 50th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at www.inchem.org/documents/jecfa/jecmono/vo41je05.htm.Accessed on Oct 17, 2017.

27. Woodward K.N. and Directorate V.M. (2017). Antimicrobial Agents. Ministry of Agriculture, Fisheries and Food, UK. Available at www.inchem.org / documents / jecfa/ jecmono/v38je04.htm.Accessed on Oct 13, 2017.