ISSN: 0975-833X

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

Vol.6, Issue 09, September - 2014

Impact Factor: SJIF : 3.845
Indexing: Thomson Reuters: ENDNOTE

ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 6, Issue, 09, pp.8829-8833, September, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

VINCRISTINE SULPHATE INDUCED SPERM SHAPE ABNORMALITIES IN MICE

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ARTICLE INFO

Article History:
Received 28th June, 2014
Received in revised form
24th July, 2014
Accepted 15th August, 2014
Published online 30th September, 2014

Key words:
Sperm-shape,
Mutagenicity,
Vincristine sulphate.

ABSTRACT

The production and classification of sperm shape abnormalities in laboratory animals through administration of chemical agents, has in recent years developed into a very reliable, species and drug specific method of assay for testing the mutagenicity of pharnacotherapeutic agent. In this paper we present our observations on the number, percentages and types of dysmorphisms induced by the intraperitoneal introduction of Vincristine Sulphate, a well-known radiomimetic, poly-functional anti-carcinogenic alkaloid, in varying dosage in Male Swiss Albino mice. In this study abnormal sperm forms ranged from 0.74 to 11.16% in treated mice, the percentage of abnormalities peaking when dose was ten times the normal for weight. The sperm shapes were of the 'amorphous' type predominantly, followed by hookless', 'banana', 'folded' and 'double head or tail' in vincristine sulphate administered animals. In very high doses the drug induced near azoospermia. This report analyses and discusses the possible pharmacokinetics of the drug on the production of the sperm shape anomalies. The observations confirm that mutagenicity of chemical agents could be tested and compared using the sperm head-shape abnormality assay method with a good degree of statistical confidence.

INTRODUCTION

Abnormal forms of spermatozoa occur in all mammals (Mann and Mann 1981). It has been demonstrated that, the fraction of dysmorphic forms of sperms could be experimentally augmented in laboratory through chemical, physical or mutagenic agents (Topham 1980; Lu and Meistrich 1979). In recent years, the application of fractional percentages of abnormal sperm types in drug assays has emerged as a reliable and specific variant in the analysis of mutagenicity of newer drug (3). In pioneering studies (Moutschen and Collizzi 1975; Wyrobeck and Bruce 1975) artificial introduction of dysmorphic spermatozoa and the typing and classification of abnormalities, doses as well as drug specificity of agents has been conclusively shown (Wyrobeck and Bruce 1978). The constancy in the percentages of types of abnormality produced by specific drug on murine sperm-shape has of late elevated this mode of study to satisfactory confidence level in drug assays. Although malformations are observed in many parts of the affected spermatozoon the 'head' shape dysmorphisms and its enumeration and classification have been accepted as the standard. The sperm head displays far less susceptibility to reaction, morphologically, than other parts of the spermatozoon to minor variations in experimental and preparation techniques. In this study we have chosen to observe the effect of a known anti-mitotic polyfunctional alkaloid, Vincristine sulphate, on the gonadal tissue products of male albino mice. This drug is used in certain leukemias (Gilliman *et al.,* 1985) in paediatric pharmacotherapeutics (Kumar 1983; Kumar 1984). The extent and range of the various abnormal forms of sperm head shape produced by varying dosages of Vincristine Sulphate are elaborated and discussed in this paper.

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MATERIALS AND METHODS

50 healthy male albino swiss mice were divided into 5 groups of animals each. Colour dyes were used as identifying markers in the different groups. Vincristine Sulphate calculated to body weight strength, 0.02 mg. was intraperitoneally injected, using doubled distilled water as dilutant and tuberculin syringe as vehicle. Group V and VI were run as controls, the former receiving only distilled water as injection and the latter was run with no interference. Group I, II, III and IV were administrated the drug in therapeutic 5 times that, 10 times 20 times dose respectively. All mice were weighed before start of experiment and thereafter on every alternative day, until say 35. The animals were sacrificed through cervical dislocation at end of the fifth week. Their reproductive tracts were bilaterally dissected out and the cauda epididymis was delivered free. The

tissue was rinsed in normal saline. The tests tissue was fixed in Bouin's solution for 24 hours. The epididymal tissue was minced, rinsed, filtered and suspended in 2ml normal saline. The cell suspension was then stained with 1% aqueous Eosin-Y for half an hour. Smear slides were prepared and observation were made using light Janavel and Zeis microscopes under green filter in 320, 400 and 500 magnifications. 1000 individual spermatozoa showing abnormal head shape were enumerated and recorded for typing. H, E and P A S stained sections of 5 micron thickness were made from the fixed testes tissues. Black and white photomicrographs were made for record, along with tabulation and graphical of results were made for analysis and comparison.

OBSERVATIONS AND RESULTS

Sperms head shape malformations formed 5.08% of total in treated Group I. the majority of abnormal forms were of the amorphous or banana type 7.04% were abnormal in group II, here also amorphous types predominated, interestingly this group II also showed a good number of double – headed and double – tailed sperms. Amorphous types were in majority in group III, the total 11.16% abnormal forms also displayed a number of doubled – heads and double tails, in Group IV the total sperms count was very low: normorphs were more in number here, though 1.54% were malformed sperms. The control groups V and VI smears accounted for abnormal sperm types within the accepted normal ranges, between 0.74% to 0.76%. (Tables 1-6 and Graphs I-VI).

Table 1. Group VI Sperm-Shape Abnormality (per 1000 spermatozoa counted)

		1	2	3	4	5	Mean
a)	Normal	986	996	990	995	993	992.6
b)	Hookless	2	2	4	1	4	2.8
c)	Banana	2	0	0	0	0	0.4
d)	Amorphous	6	2	5	4	3	4.0
e)	Folded	0	0	1	0	0	0.2
f)	Double headed or tailed	0	0	0	0	0	0.0
g)	%Abnormality	1.1%	0.4%	1.0%	0.5%	0.7%	0.74%

Table 2. Group V Sperm – Shape Abnormality (per 1000 sperms counted)

		1	2	3	4	5	Mean
a)	Normal	970	958	944	896	976	949
b)	Hookless	2	3	3	6	4	3.6
c)	Banana	0	1	0	0	1	0.4
d)	Amorphous	3	4	1	6	4	3.6
e)	Folded	0	0	0	0	0	0.0
f)	Double headed or tailed	0	0	0	0	0	0.0
g)	%Abnormality	0.6%	0.8%	0.4%	1.2%	1.0%	0.78%

Table 3. Group I Sperm Shape Abnormalities (per 1000 sperms counted)

		1	2	3	4	5	Mean
a)	Normal	970	948	944	896	978	949
b)	Hookless	5	7	10	26	7	11
c)	Banana	14	10	16	21	4	13
d)	Amorphous	8	15	26	52	9	22
e)	Folded	3	8	4	4	2	402
f)	Double headed or tailed	0	2	0	1	0	0.6
g)	%Abnormality	3.0%	4.2%	5.6%	10.4%	2.2%	5.08%

Table 4. Group II Sperm Shape Abnormality (per 1000 sperms counted)

		1	2	3	4	5	Mean
a)	Normal	962	913	908	941	924	929.8
b)	Hookless	4	9	14	10	15	10.4
c)	Banana	6	20	19	7	6	11.6
d)	Amorphous	16	43	38	29	46	34.4
e)	Folded	7	6	16	7	4	8.0
f)	Double headed or tailed	5	9	5	6	5	6.0
g)	%Abnormality	3.8%	8.7%	9.2%	5.9%	7.6%	7.04%

Table 5. Group III Sperm Shape Abnormality (per 1000 sperms counted)

	1	2	3	4	5	Mean
a) Normal	865	902	884	911	880	888.4
b) Hookless	20	31	25	24	28	25.6
c) Banana	36	17	19	15	22	21.8
d) Amorphous	57	31	54	32	51	454.0
e) Folded	14	10	10	10	9	11.0
f) Double headed or tailed	0	0	0	0	0	0
g) %Abnormality	13.5%	9.8%	11.6%	8.9%	12.0%	11.16%

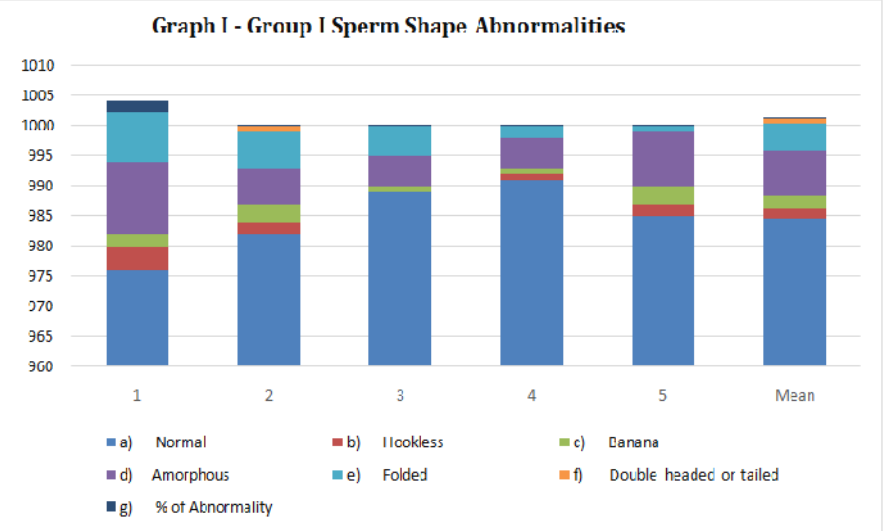
Table 6. Group IV Sperm Shape Abnormality (per 1000 sperms counted)

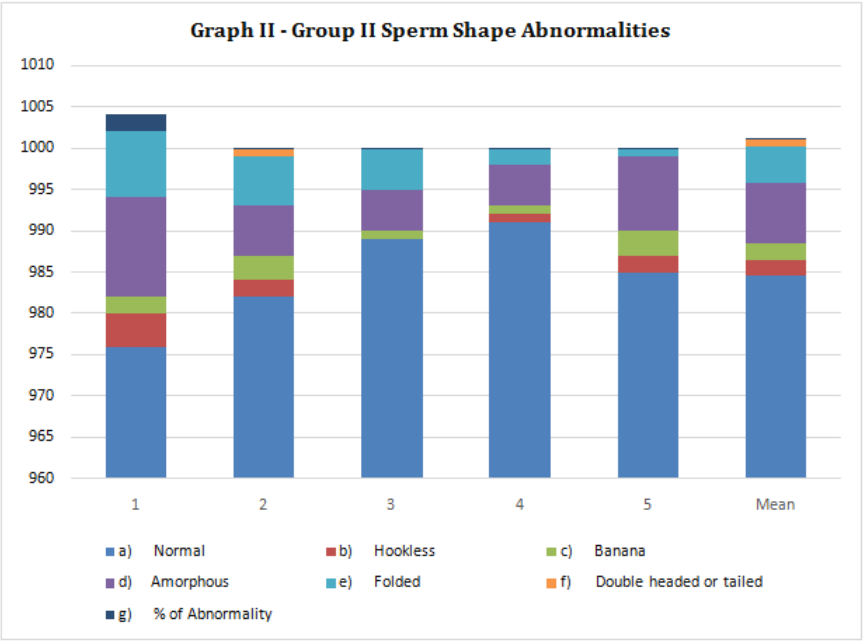
	1	2	3	4	5	Mean
a) Normal	976	982	989	991	985	984.6
b) Hookless	4	2	0	1	2	1.8
c) Banana	2	3	1	1	3	2.0
d) Amorphous	12	6	5	5	9	7.4
e) Folded	8	6	5	2	1	4.4
f) Double headed or tailed	0	1	0	0	0	1.0
g) %Abnormality	204%	1.8%	1.1%	0.9%	1.5%	1.54%

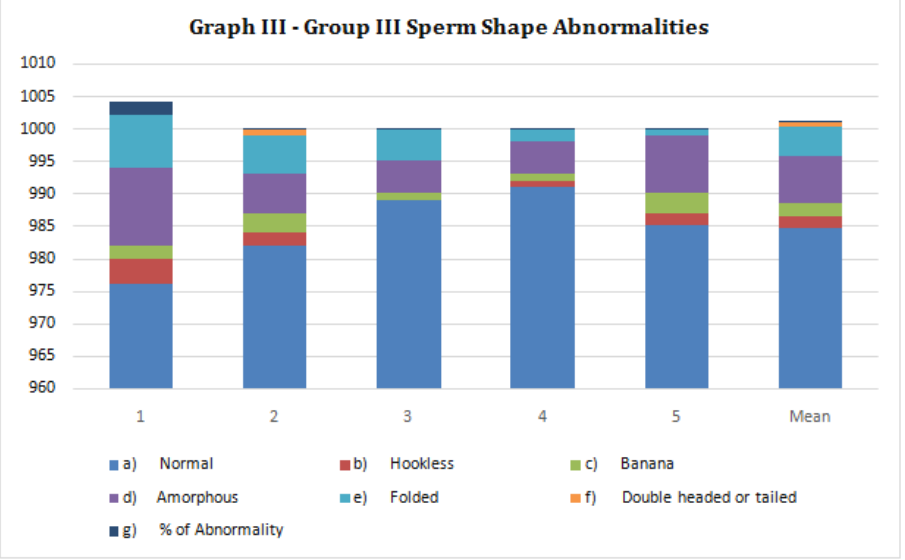
Loss of weight of mice occurred from day 2 onwards upto day 8, after which there was a gradual increase in weight in Group I, II and III. In Group IV however, the weight loss was progressive. This group showed massive mortality and had to be re-run in order to obtain statistics of any significance. The combined weight of tests also lost an average of 0.112 gms. From a dimension of 7 X % mm in healthy mice the drug administered specimens had testicles of only 5 X 3 mm.

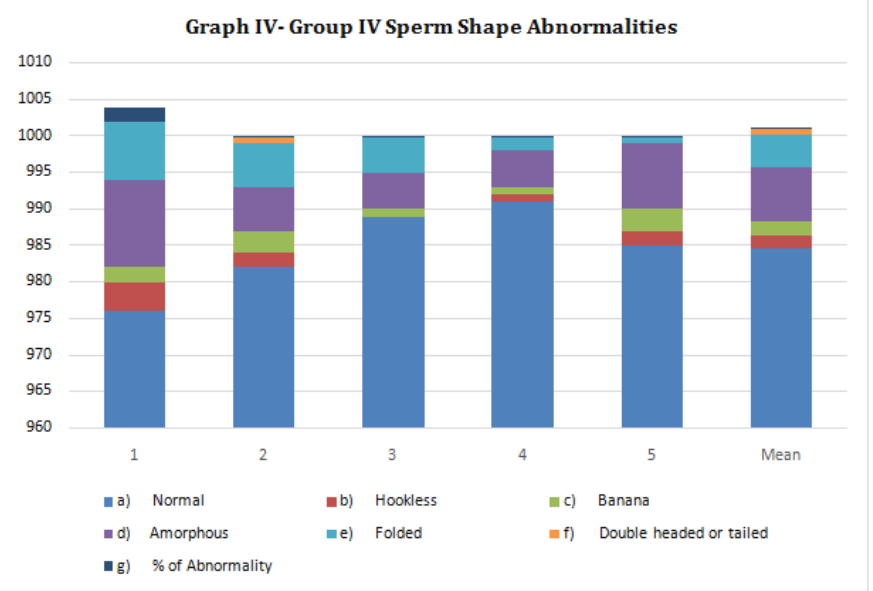
DISCUSSION

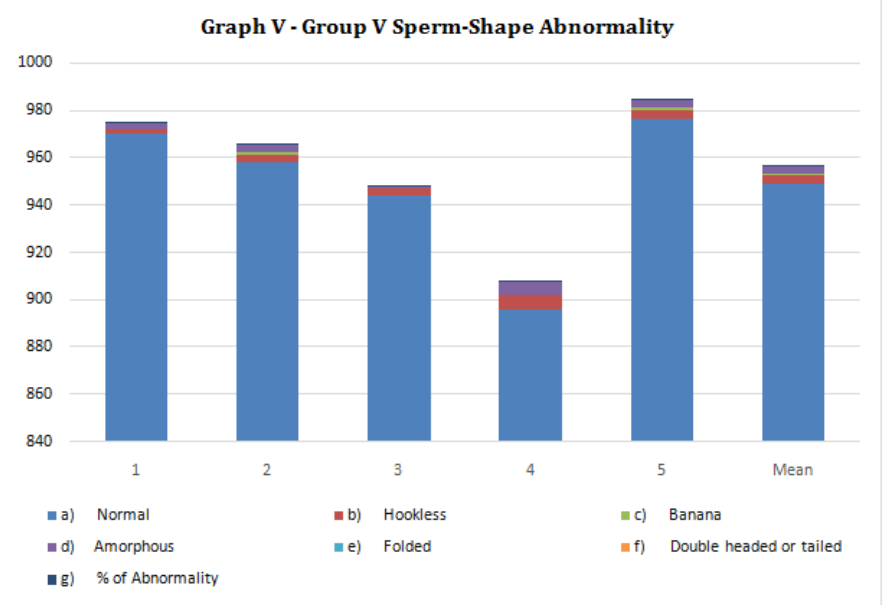
Research into the genesis and effect of sterility forms the basis of the science of reproductive biology and medicine (Wyrobeck and Bruce 1978). The population dynamics and its influence on social and community problems, make for a deeper understanding of the processes of cogenesis and spermatogenesis, and the factors controlling them, imperative. Sperm count fecundity and viability are important factors determining the outcome of in – vitro fertilization and insemination procedures. The biomechanisms that facilitate sperm production, motion and function are varied and complex. Chemical factors are known to influence all the aforementioned. The stage by stage histogenesis, the time specificity of sperm maturation and the periodic regularity with which the gonads perform, make study of interference through insults stage specific enough to warrant scientific attention. This chemical – spermatogenesis interaction has rendered studies on drug induced sperm shape abnormalities of having vital bearing; moreover the spin off from these methods of analysis, has led to a burgeoning new area in mutagen study

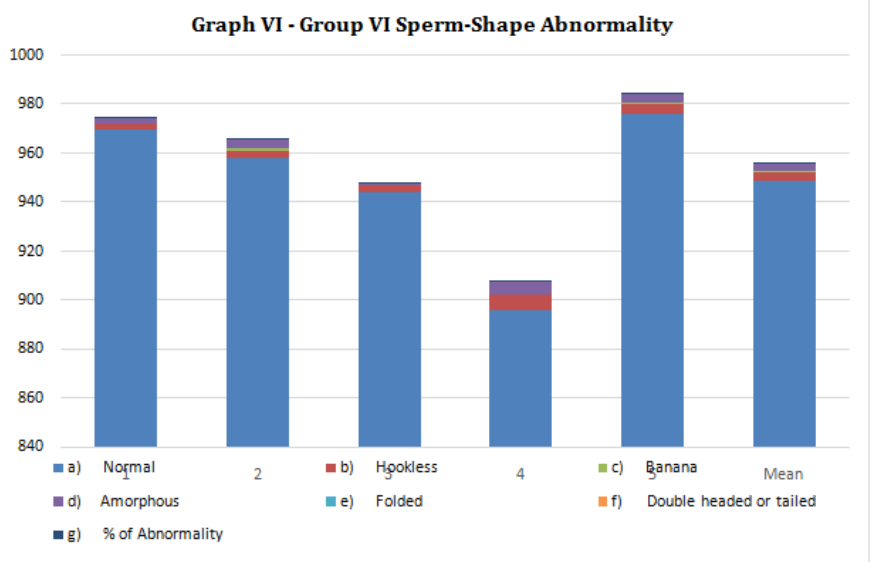

Graph I - Group I Sperm Shape Abnormalities
1010
1005
1000
995
990
985
980
975
970
965
960
1
2
3
4
5
Mean
a) Normal
b) Hookless
c) Banana
d) Amorphous
e) Folded
f) Double headed or tailed
g) % of Abnormality


Graph II - Group II Sperm Shape Abnormalities
1010
1005
1000
995
990
985
980
975
970
965
960
1
2
3
4
5
Mean
a) Normal
b) Hookless
c) Banana
d) Amorphous
e) Folded
f) Double headed or tailed
g) % of Abnormality


Graph III - Group III Sperm Shape Abnormalities
1010
1005
1000
995
990
985
980
975
970
965
960
1
2
3
4
5
Mean
a) Normal
b) Hookless
c) Banana
d) Amorphous
e) Folded
f) Double headed or tailed
g) % of Abnormality


Graph IV- Group IV Sperm Shape Abnormalities
1010
1005
1000
995
990
985
980
975
970
965
960
1
2
3
4
5
Mean
a) Normal
b) Hookless
c) Banana
d) Amorphous
e) Folded
f) Double headed or tailed
g) % of Abnormality


Graph V - Group V Sperm-Shape Abnormality
1000
980
960
940
920
900
880
860
840
1
2
3
4
5
Mean
a) Normal
b) Hookless
c) Banana
d) Amorphous
e) Folded
f) Double headed or tailed
g) % of Abnormality


Graph VI - Group VI Sperm-Shape Abnormality
1000
980
960
940
920
900
880
860
840
1
2
3
4
5
Mean
a) Normal
b) Hookless
c) Banana
d) Amorphous
e) Folded
f) Double headed or tailed
g) % of Abnormality

assays using sperm shape abnormality fraction percentage, with its drug dose and species speciality constancy, of commanding value in drug testing procedures (Avadhani 1991; Kumar 1983; Rahiman *et al.*, 1988).

The effects of Vincristine sulphate on sperm shape abnormalities and their causative mechanisms are elaborated here. Derived from vinca alkaloids, vincristine is an antiproliferative, radiomimetic anti – carcinogenic drug. The drug arrests cell growth through its effect on cytoskeletal elements and inhibits spindle formation - essential for normal cell division. Besides, by selectively binding to the protein tubulin, the drug prevents cell shape change (Avadhani 1991) and disrupts axoplasmic flow (Kumar 1983). In the rapidly diving cells of the gonads, vincristine inhibits spermatogonial function to a pronounced degree. The products of spermatogenesis too are not spared the pharmacokinetics of the alkaloid. Acrosomal head shape of sperm is disrupted from the normal by affecting the tubulin polymerisation into microtubules and by inhibiting axoplasmic flow (Avadhani and Kumar 1994). Vincristine disrupts ciliary action and check motility of spermatozoa. This study clearly establishes that the drug produces a wide range of sperm shape abnormalities, depending on the dose of administration. Amorphous forms, followed by hookless and banana types constituted the major of the abnormal forms induced by vincristine sulphate.

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