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Effects of medroxyprogesterone - acetate and dihydrotestosterone combination in male contraception

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Abstract

Effects of weekly intramuscular injections of nonaromatic Dihydrotestosterone (DHT, 2mg/kg body wt.) and Medroxyprogesteroneacetate (MPA, 10mg/kg body wt.) for 60 days were observed with respect to spermiograms, reproductive organ and fertility in adult male rats (*Rattus rattus norvagicus*, Charles Foster strain). Sperm count, sperm motility and viability from the cauda epididymis showed a significant reduction. The sperm acrosomal enzyme such as acrosin and hyaluronidase also reduced leading to loss of their function. The superoxide dismutase (SOD) levels also dropped indicating sperm membrane modification and subsequent correlated to loss of their morphology. As a result of these effects, the treated animals had a poor fertility rate. Testis spermatogenesis was affected. Cauda epididymis milieu was also altered. However, hematological parameters revealed no changes in blood cell counts and hemoglobin levels. The clinical chemistry data also indicated no alteration in transaminases. Cholesterol, SOD and protein levels in rats treated with MPA + DHT for 60 days. Libido of these animals unchanged as serum testosterone was within normal range. Ninety days after cessation of the treatment, the reproductive function of these animals was comparable to normal animals. Therefore this combination would be useful for induction of functional sterility by suppressing sperm functions in the male strain with proven fertility weighing from 250-300 g when used for the experiments.

Keywords: male contraception, steroidal hormones, spermatogenesis, hematology.

Introduction

Hormones control the spermatogenesis in male hence contraception in males can be exploited via a combination of sex hormones and its allies. The combination of various steroids have been tried for interfering the process of sperm formation by endocrinological mechanisms on the target organ (Wu and Aitken, 1989; Puri and Van Look, 1994). Steroids alone or in combinations create oligospermia or azoospermia or bring about deformations in the sperm rendering them unfertile. This mechanism is fairly exploited in male contraception. Moreover this chemical castration is quite reversible and hence very important if it is put to use for human male contraception. Also the chemicals used should not have psychological influence on the masculinity say libido mechanism. In this study a combination of MPA+DHT was used on rats as the effects of these steroids on tissue and sperm metabolism are not very clearly reported.

Materials and Methods

1. Maintenance of animals:

Albino rats were caged in air-conditioned animal house and maintained on standard chow and water *ad libitum*. Animals of different experimental groups were caged separately and a maximum of four animals

per cage was maintained. These animals were mainly divided into two groups. To one group of animals weekly intramuscular injections of MPA (10mg/kg body wt.) and DHT (2.0mg/kg body wt.) in olive oil was given for a period of 60 days . Out of these treated animals some animals were kept for withdrawal study for 60 and 90 days. Control groups received only olive oil. The hormones were purchased from Sigma Chemical Co., USA. After the completion of the treatment, both the treated and control animals were autopsied and tissue were weighed. The epididymal tissue were utilized for the preparation of sperm suspension according to the method of Winner *et al*. (1971) and other biochemical parameters. The testis and epididymal tissue were utilized for bio chemical estimation. The blood was collected by cardiac puncture and serum was separated and stored at -20° C for further analysis.

2. Spermiogram and fertility rate:

In spermiogram, sperm count and motility were done using haemocytometer (Prasad *et al.* 1972). The sperm viability was assessed with Trypanblue stain(Talbot and Chacon, 1861). Sperm morphology was assessed using eosin stain (Pandey *et al.,* 1990) fertility test was performed according to WHO protocol MB-50. Briefly, the cyclic females in proestrous or estrous were cohabited with treated males in a ratio of 2:1.Next day morning mating was confirmed by the presence of sperms in the vaginal smear.

On the 16th day females were autopsied and the uteri were exposed for the presence of implantation sites. Fertility tests was positive if the implantation sites were observed and negative in their absence.

3. Biochemical parameters:

In sperm the superoxide dismutase (SOD) [EC. 1.15.1.1] Kakkar*et al.* 1984, and the sperm acrosomal enzymes such as hayaluronidase [EC. 3.2.1.35] and acrosin [EC. 3.4.21.10] activity were done (using BAEE as a substrate Polakoski and Zaneveld, 1977). The biochemical tests such as succinate dehydrigenase [EC.1.3.9.8] adenosine triphosphatase (ATPase) [EC. 3.6.1.3] acid phosphatase [EC. 3.1.3.2], hydroxysteroid dehydrogenase (HSDS), sialic acid, protein and cholesterol were estimated using standard bio chemical methods. (Beatty *et al*. 1966, Quinn and White, 1968, Bassey *et al.* 1946, Talalay 1962 , Jordian *et al.* 1971 , Lowry *et al.* 1951, Pearson *et al.,* 1953).

4. Haematological and serum parameters:

The haematological parameters such as blood cell counts and haemoglobin contents were carried out usinghaemocytometer and haemoglobinometer respectively. The sereum level of glutamic pyruvate transaminase (GPT) [EC. 2.6.1.2] and glutamate oxaloacetate transaminase (GOT)[EC.2.6.1.1] (Reitman and Frankel, 1957). Cholesterol and protein were also estimated. The radio immune assay (RIA) of testosterone was performed using RIA-kits purchased from Amar Diagnostics, Bombay. The data for all biochemical estimations were subjected to statistical analysis using student T-test, a significant level of P<0.05 was accepted.

Results

1. Gravimetric spermiograms and fertility rate:

Body weight did not change in all the experimental groups, similarly nosignificant reduction was noted in organ weights of the epididymis and vas deferens by MPA + DHT, regimen.

Accept in the testes where the decrease was significant (Table-1) but it recovered gradually after 60 and 90 days of withdrawal of the treatment. The caudaepidimal sperm count, sperm motility and viability showed marked alterations in the treated animals.

TABLE-1: Body and organ weights of control and experimental animals

Parameters	Control	MPA + DHT Treated	60 Days Recovery	90 Days Recovery
Body weight (gm)	285± 3.2	276 ±2.2	276 ±2.8	278 ±3.2
Testis weight (mg)	1316 ± 4.3	737±3.4*	950 ±1.6	1251 ± 5.4
Caput epididymis(mg)	227±1.8	183 ±1.1	192 ± 1.5	223 ±0.9
Cauda epididymis(mg)	214 ±1.3	169 ± 1.5	187 ± 1.3	206 ± 1.6
Vas deferens (mg)	110 ± 1.2	95 ±1.2	1.3 ±0.9	109 ± 1.1

Values are mean ±- S.E. * P<0.001

A gradual recovery was observed in this caudaepidimal sperm profile after the cessation of injection of first 60 and 90 days (Table-2) all these finding led to a reduction in the fertility rate. However the mating rate of the animals was not affected. But their fertility rates and litter size were significantly reduced (Table-3). No significant variation was noted in serum testosterone levels (Table-4).

TABLE-2: Caudaepididymal sperm profile of control and experimental animals

Parameters	Control	MPA + DHT Treated	60 Days Recovery	90 Days Recovery
Sperm motility (%)	72	18	52	68
Sperm count (million/100 mg)	86.5 ±2.3	15.1±0.57*	58.1±1.8	76.7±0.9
Sperm viability:				
Live%	84	18	58	80
Dead %	16	82	42	20
Abnormal forms (%)	3-6	34	14	3
Decapitated forms(%)	5-7	30	12	4

Values are mean ± S.E. * P<0.001

TABLE-3: Fertility rate and serum testosterone levels of control and experimental animals

Parameters	Control	MPA + DHT Treated	60 Days Recovery	90 Days Recovery
Mating rate (%)	84	75	80	82
Fertility rate in mated animals (%)	100	18	64	94
Litter size	9±0.4	2.1±0.1	5.8±0.6	7.7±0.6
Serum testosterone	3.4±0.05	4.0±0.03	2.4±0.03	3.3±0.04

Values are mean ± S.E. * P<0.001

TABLE-4: Acrosomal enzymes and SOD of caudaepididymal spermatozoa of control and experimental animals

Parameters		Control	MPA + DHT Treated	60 Days Recovery	90 Days Recovery
Hyaluronidase *		242	113.6±1.8	183.1±1.5	229.9±1.4
Acrosin	Free acrosin**	26.4±0.6	$12.3±0.9^{+}$	19.4±0.2	24.2±0.7
	AAI **	15.3±0.2	$5.49±0.2^{+}$	12.4±0.3	14.6±0.3
	Proacrosin **	10.3±0.7	$8.64±0.7^{+}$	9.49±0.6	10.1±0.3
Superoxide dismutase (SOD) (units/10^6 sperms)		0.55±0.04	$0.32±0.05^{++}$	0.46±0.05	0.53±0.03

Values are mean ± S.E. * P<0.001, **P<0.01
*n moles of N-acetyl glucosamine/10^6 sperm/hour.
**n moles of BAEE hydrolysed /10^6 sperm/minute.
AAI= acrosion-acrosin inhibitor complex.

2. Biochemical studies:

Caudaepidymal sperm biochemical parameters such as superoxide dismutase (SOD), hyaluronidae, and acrosin activities were declined significantly by this treatment. Withdrawal of the treatment for 60 and 90 days, this enzyme levels restored to the normal level (Table-5), epidermal enzyme activities viz., SDH, and ATPase were reduced significantly in MPA +DHT treated rats. Sialic acid was also declined. But protein was unchanged in all the experimental groups (Table-5). Similarly in the testis of the treated animals a decrease was noticed in the 3B and 17B hydroxysteroid dehydrogenase (HSDS), SDH and ACPase activities. Protein and cholesterol levels remained unaltered by these injections. All this effects were found to be restored after 90 days of recovery-(Table-5-6).

TABLE-5: Caudaepididymal tissue biochemical parameters of control and experimental animals

Parameters	Control	MPA + DHT Treated	60 Days Recovery	90 Days Recovery
Succinate dehydrogenase	428±5.8	245±7.5^{+}	386±6.6	412±3.4
Adenosine triphosphatase	31.7±1.16	23.9±1.09^{+}	27.5±1.21	30.4±0.96
Protein (mg/100mg tissue wt.)	16.5±0.6	15.3±0.3	15.8±0.3	16.5±0.2
Sialicacid (µg/mg tissue wt.)	4.15±0.09	2.71±0.06$^{\pm}$	3.55±0.08	3.85±0.09

Values are mean ± S.E. * P<0.001
*µgmformazan formed /15 min/100 mg tissue wt.
**µ moles of ip released /30min/100 mg tissue wt.

TABLE-6: Testicular biochemical parameters of control and experimental animals

Parameters	Control	MPA + DHT Treated	60 Days Recovery	90 Days Recovery
3 HSD*	0.6235±0.004	0.4426±0.005^{++}	0.4830±0.004	0.5898±0.008
17 HSD*	0.2419±0.005	0.0991±0.008^{++}	0.2204±0.006	0.2388±0.006
Succinate dehydrogenase **	418.4±2.6	254.5±3.3***	379.9±2.4	410.4±2.4
ACPase^{+}	0.74±0.07	0.44±0.06	0.65±0.03	0.71±0.06
Protein (mg/100 mg tissue wt.)	18.5±0.9	16.6±0.8	17.4±0.8	18.3±0.5
Cholesterol (mg/100 mg tissue wt.	0.394±0.04	0.371±0.03	0.383±0.04	0.392±0.05

Values are mean ±- S.E. *** P<0.001, $^{++}$P<0.01
*n moles 5 -Diol formed /mg protein/30 minutes. ,**µ g Formazan formed /15min./100mg tissue wt.
$^{+}$µ moles of p-Nitrophenol released /30 min/100 mg tissue wt., HSD= Hydroxy Steroid Dehydrogenase

3. Toxicological study:

The toxicological study revealed no significant changes in the Hemoglobin content and blood cell counts (Table-7), similarly no changes were observed in serum levels of protein, cholesterol as well as in the SOD activity in the blood however an insignificant increase in the serum glutamate pyruvate transaminase (SGPT) and serum oxaloacetate transaminase (SOT) were observed in the treated animals but where within the normal range the observed changes were reversible after withdrawing the treatment (Table-8).

TABLE-7: Histological parameters of control and experimental animals

Parameters	Control	MPA + DHT Treated	60 Days Recovery	90 Days Recovery
Haemoglobincontent(gm%)	15.3±0.14	17.1±0.20	16.4±0.20	15.5±0.33
RBC Count (million/mm^3)	7.46±0.26	8.04±0.20	7.71±0.19	7.42±0.11
WBC Count (thousand/mm^3)	5749±21	6520±19	6010±26	5906±17
SOD (units/ml)	25.50.83	23.6±0.77	24.5±0.61	25.4±0.44

Values are mean ± S.E.

TABLE-8: Serum parameters of control and experimental animals

Parameters	Control	MPA + DHT Treated	60 Days Recovery	90 Days Recovery
Serum Protein (mg/ml)	64.5±2.49	66.8±2.51	65±2.21	64.6±2.22
Serum Cholesterol(mg/ml)	0.94±0.02	1.06±0.01	1.01±0.03	1.04±0.04
SGOT (mU/ml)	72±2.84	82±2.84	77±1.91	69±1.77
SGPT (mU/ml)	28±1.10	34±1.11	31±0.33	29±0.91

Values are mean ± S.E.
SGPT= serum glutamate pyruvate transaminase
SGOT= serum glutamate oxaloacetate transaminase

Discussion

The study describes the reversible contraceptive effects of a hormonal combination of MPA and non aromatisable androgen DHT in rats. This treatment had no effect on body weights. But reduction in the testis weight was about 40% and this reduction was attributed to the loss of spermiogenic elements as a result of androgen deprivation. Other organ weights did not vary significantly. Further the data showed a reduction in the fertility of treated animals. This effect was further evidenced by the reduction in sperm counts of cauda epididymis in treated rats. Sperm motility was also declined markedly after this steroid injection to rats. Numerous hormonal treatments in alone and in combinations, suppressed sperm motility in animals and man (Wu and Aitken, 1989; Puriand Van Look, 1994; Roy 1994; WHO;1994). The decline in the sperm viability has been correlated with an alteration in sperm membrane permeability. This was further substantiated by a reduction in SOD in this study. The enzyme depletion is related to an accumulation of more reactive oxygen species. Which have detrimental effect on sperm membrane (Rao and Roy, 1992). The abnormal sperm morphology included were decapitation, acrosomal defects agglutination and other anomalies were noted. The sperm acrosomal enzymes hyaluronidase and acrosin revealed a fall in their activities by MPA+DHT treatments indicating the loss of acrosomal function. Hence, these sperms were unable to fertilize the ova. Similarly MPA+DHT treated human spermatozoa are unable to penetrate the hamster oocytes supporting our data (Wu and Aitken, 1989). The enzyme activities of SDH and ATPase in the epididymis were declined as a result of local androgen deprivation caused by this hormonal treatment. Decline in the sialic acid level indicated its altered microenvironment which is normally under control of androgens (Robaire and Hermo, 1988). Protein was unchanged. The testicular biochemical parameters such as SDH, ACPase and hydroxysteroid dehydrogenase (HSDS) activities were also reduced by the MPA+DHT injections in addition to inhibition of spermiogenesis process. This could be explained by a probable reduction in the intramuscular androgen levels. But these effects were restored after withdrawing the treatment for 60 and 90 days gradually. DHT alone also effects testicular function (Ramkrishnan *et al.*, 1989).

The serum parameters such as testosterone, SGPT,SGOT, cholesterol and protein were not altered very significantly. Similarly the blood cell counts and hemoglobin levels were also within the normal range. Therefore this combination did not produce any toxic effect on the biochemical profile of blood. These data were comparable to those observed with other steroid combinations (Rao and Roy, 1994).

Thus it is obvious that this combination generated reversible contraceptive effect. At the same time the side effects were minimum and transient. Hence this steroid combination is useful for induction of functional sterility in males without side effects.

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References

1. **Betty C.B., Bessinger G.M., Dully C.C. and Boceck R.M. 1966.** Comparison of red and white voluntary skeletal muscles of several species of primates. *J,Histochem*,**14:**590
2. **Bessey O. A., Lowry O.H. and Brick N.H. 1946.**A method for the determination of acid and alkaline phosphatase with 5 cu mm of serum. *J. biol. Com.* **164:**121
3. **Hndelsman D.J. 1994.**Hormonal male contraception. In: current concepts in fertility regulation and reproduction. Puri C. P. and Van Look, PFA(eds.) Willey Eastern Pub., New Delhi.
4. **Jordian G.W., Dean L. and Roseman S. 1971.**Periodate resorcinol methods for quantitative estimation of free sialic acids and their gycosides , *J. biol. Chem.* **246:**430
5. **Kakkar P., Das B. and Vishvanathan P. N. 1984.**A modified spectrophotometric assay of superoxide dismutase, *Ind. J. of Bio. Chem. Bio.Phy*. **21:**30
6. **Lowry O.H., Rosebrough N.J. Farr A. L. and Randall R.J. 1951.**Protein measurement with Folinphenol reagent, *J. Biol. Chem*. **193:**265
7. **Mann P. and Lutwak Mann C. 1981.**Male reproductive functions and semen, Springer Verlag, Berlin.
8. **Matsumoto A.M. 1988.** Is high dosage testosterone an effective male contraceptive agent?*Fertil***.** *Steril***. 50:**324
9. **Nieschlag E. and Behre H.M. 1990.**Experimental use of testosterone in the development of a male contraceptive. In: testosterone action deficiency substitution. Nieschlag E. and BehreH.M. (eds.) Heidelberg. Springer Verlagpp. 106
10. **Nieschlag E. and Habenicht U.F. 1992.** Spermatogenesis, fertilization, contraception, molecular, cellular and endocrine events in male reproduction. Springer Verlag, Berlin**.**
11. **Pandey N., Gundevia F., Prem A.S. and Ray P.K. 1990.** Studies on the genotoxicity of endosulfan an organochlorine insecticide in mammalian sperm cells.*Mutation Research***: 242:** 1-7
12. **Pearson S., Stern S., and Mc. Gavak T.H. 1953.**A rapid accurate method for the determination of total cholesterol in serum. *Analytil. Biochem*. **25:**813
13. **Polakoski K.L. and Zaneveld L.J.D. 1977.** Biochemical examination of the human ejaculate. In: ESE Hafez(ed.) Techniques of Human Andrology, Biomedical press, Elsevier, North Holand.pp280
14. **Prasad M.R.N., Chinoy N.J. and Kadam K.M. 1972.**Changes in succinate dehydrogenase levels in rat epididymis in undernormal and altered physiological conditions. Fertile. Steril. **23:**186
15. **Puri C.P. and Van Look P.F.A. 1994.**Current concepts in fertility regulation and reproduction, Willey Eastern Ltd. New Delhi, India.
16. **Quinn P.J. and White I.G. 1968.**Distribution of adenosine triphosphatase in rat and bull spermatozoa, *J. of Reproductive Fertility*, **15:**449
17. **Rajlaxmi M. 1994.** Induction of azoospermia by hormonal methods of non-human primates, In: Current Concept of Fertility Regulation and Reproduction Puri C.P. and Van Look P.F.A.(eds.) Willey Eastern Ltd. New Delhi. pp. 117
18. **Rao M.V. and Roy G.K. 1993.**Biochemical and morphological changes of spermatozoa in progestin-androgen injected rats. *Ind. J. of Exp. Biol*. **31:**12
19. **Ramakrishnan P.R., Kaur J., Rajlakshmi M. 1989.**Effects of dihydrotestosterone on testicular and accessory gland function in male rhesus monkey. *Contraception* **40:**111
20. **Reitman and Frankel S. 1957.**A colorimetric method for the determination of serum glutamic oxaloacetate transaminase and serum glutamate pyruvate transaminase. *J. of Clinical Patho*.**28:**56
21. **Robaire and Hermo L. 1988.**Efferent ducts, epididymis and vasdefferns: structure function and their regulation. In: Physiology of Reproduction, Knobil E.*et al.* (eds.) Raven Press NY. pp999
22. **Roy G.K. 1994.**Studies on mammalian spermatozoa in relation to fertility regulating agents. Ph.DThesis Gujarat University, Ahmedabad, India**.**
23. **Talbot and Chacon R.S. 1981.** A triple stain technique for evaluation of normal acrosome reaction of human sperm. *J of Exp. Zool*. **215:**201
24. **Talalay P. and Benson A. M. 1962.**3-keto steroid isomerase, In: The Enzymes Boyer P.D.(ed.) Academic Press NY, **6**:591
25. **WHO. 1983.** MB-50 a method for examining plant extract administered orally on the fertility of male rats.Ap/Ip, **9914**, E**.**

26. **WHO. 1994.** Challenges in reproductive health, Research Biannual Report, Geneva, Switzerland.
27. **Winner D.A., Mikowitch and Winner M.B.N. 1971.**Hormonal effects on the gonad lactate dehydrogenase, FEBS letters **16:** 21
28. **Wu. F.C.W. and Aitken R.J. 1989.**Suppression of sperm function by depot medroxyprogesterone acetate and testosterone enanthate in steroid male contraception**.** *Fertil. Steril***. 51:**691.

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