MELATONIN EXERTS STIMULATORY EFFECT ON HUMAN SPERM MOTILITY, *IN VITRO*

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SUMMARY: Pineal indolic compounds have been postulated to play an antigonadotrophic role in the reproductive system of mammals. In humans, indoleamines are localized in tissue fluids such as plasma, serum, cerebrospinal fluid, aqueous humor, semen, and follicular fluid. Because they exhibit antigonadotrophic properties, we examined whether melatonin causes any inhibitory effects on sperm motility. In the present study, significant stimulation of sperm motility by this indole was observed. Furthermore, the presence of melatonin in incubation medium did not decrease, but on the contrary increased sperm velocity slightly and nonsignificantly. These data raise the possibility that the presence of high doses of indoles in reproductive fluids may increase sperm motility and velocity. This stimulatory effect of melatonin on sperm motility parameters was concluded to be due to its action as a highly efficient free radical scavenger and general antioxidant.

Key Words: In vitro, melatonin, indoleamines, sperm motility, antioxidants.

INTRODUCTION

The pineal secretory product named melatonin is an indole, and it has some effects on the gonads varying markedly from species to species (1). The influence of indoleamines on the neuroendocrine reproductive axis of a variety of experimental animals is well documented and they are postulated to play an antigonadotrophic role in the reproductive system of mammals (1,2). Although the functions of melatonin and the pineal in humans remain obscure, there are observations suggesting that it is involved in regulating the release of pituitary hormones and other endocrine functions (3,4). Since the pineal indolic compounds have been extensively reported to inhibit some secretions or actions of hormones related to reproduction, the effects of melatonin (N-acetyl-5-methroxytryptamine) were studied on some sperm motility parameters of normozoospermic samples (2,5-9).

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MATERIALS AND METHODS

This study was performed with samples of semen from 24 men undergoing semen analysis within the andrology division of ,Çukurova Medical Faculty, Department of Urology. Their ages varied between 25 and 45 years (mean±SE 31.4 ± 0.99),

and all were normozoospermic (sperm concentration ≥20x10^6/mL) according to World Health Organization criteria. Semen was collected by masturbation into sterile plastic containers. Of the 24 normozoospermic samples, 15 were asthenozoospermic (motility < 50%) and one was teratozoospermic (abnormal forms > 50%). Average-path velocity (VAP) was normal in all samples (sperm velocity > 25μm/s). Fresh ejaculates were allowed to liquefy at room temperature for 20-30 min and motile spermatozoa were obtained by centrifuge/swim-up migration. Ham's F10 solution (Sigma) was used as the incubation medium and included (10%) heat inactivated fetal cord serum. For the swim-up procedure, 2 mL of each semen sample was washed twice in 3 mL of Ham's F10 solution and centrifuged at 1000 rpm for 10 min. The final pellet was encubated for swim-up in a CO_2 incubator at 37°C and after 1 h incubation, spermatozoa were recovered in the supernatant. An assessment of initial % motility, % progressive motility and sperm velocity was performed by computer-assisted sperm analysis (CASA) technique (Hamilton Thorn Research, Danvers, USA). Each washed specimen was divided into two 100 μL aliquots.

Melatonin was dissolved in absolute ethanol and diluted with Ham's F10 solution (1 mg / 25 mL). Melatonin was added to one of the aliquots at final concentration of 200 pg/mL. The second aliquot added only Ham's F10 in equal volume, served as the control. These samples were incubated in a CO_2 incubator at 37°C for 2 h. At the first and second hours of incubation, % motility, % progressive motility and sperm velocity were assessed by CASA technique. Statistical analysis was performed by student's t-test. Results were expressed as the mean ± SE, and a probability < 0.05 was accepted as significant.

RESULTS

Significant increases in % motility in the first and second hours ($p < 0.01$) and % progressive motility ($p < 0.05$ in the first, and < 0.001 in the second hours) occurred when the spermatozoa were incubated with melatonin (Table 1, Figures 1-2). A slight increase in the mean velocity was also determined in the melatonin-incubated group, but this change was nonsignificant ($p > 0.05$; Table 1).

This study demonstrated also that when motile spermatozoa obtained by swim-up migration are incubated for 1 or 2 h more either with or without melatonin, no further recovery is seen in the sperm motility parameters (Table 1, Figures 1-2).

DISCUSSION

Being contrary to other findings in the literature, this study demonstrates the stimulatory effect of melatonin, which improved sperm motility significantly. The association between the pineal gland and reproductive function has been speculated since 1878, shown by evidence in experimental animals (10). In 1968, Wurtman *et. al.* published the first data demonstrating a negative influence of exogenous melatonin on mammalian reproduction (11). Additionally, Mas *et. al.* found a negative effect of melatonin on the prostatic and seminal vesicles of rats and suggested melatonin as a potential inhibitor of spermatogenesis (12). The pineal

Table 1: Mean (±SE) % motility, % progressive motility and velocity for 24 semen samples after incubation with and without melatonin.

	Time (hour)				
	0[a]	1*	2*	1**	2**
Motility (%)	79.04 ± 3.21	67.38 ± 4.17	56.54 ± 4.11	72.04 ± 4.18[b]	63.8 ± 4.12[b]
Progressie motility (%)	50.29 ± 2.92	41.25 ± 3.81	31.04 ± 3.19	46.79 ± 3.53[b]	40.38 ± 2.96[b]
Sperm velocity (μm/s)	45.04 ± 1.33	43.58 ± 1.76	41.96 ± 1.87	45.21 ± 1.99	43.04 ± 1.99

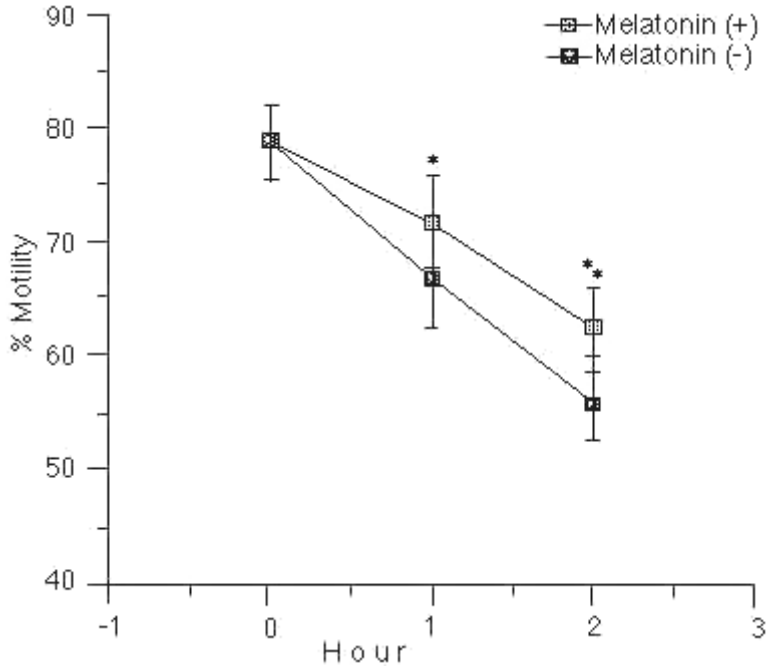
(*) without melatonin,

(**) with melatonin,

(a) motile spermatozoa obtained initially from semen by swim-up migration,

(b) differences are significant when compared to control samples,incubated without melatonin.

Figure 1: Mean (±SE) % motility of normal spermatozoa, when incubated with and without melatonin (*; differences are significant when conpared to control samples incubated without melatonin).

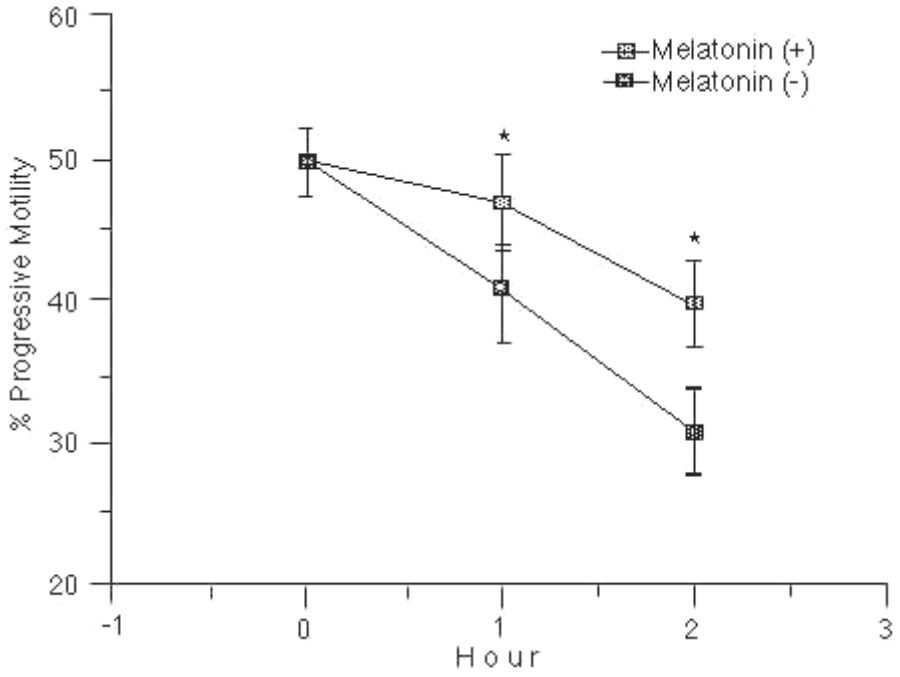


indole, melatonin, has been reported to act is an antigonadotrophic hormone, inhibiting the release of pituitary gonadotrophins and spermatogenesis (7,13). In 1992, time and dose-dependent inhibition of sperm motility by indoleamines was observed, *in vitro* (9).

Many studies have been performed to determine the site of action of melatonin. Direct cellular effects of melatonin have been demonstrated in the hypothalamus, pituitary gland, gonads and pineal gland *in vitro* (14). In the recent years, identification and characterization of a high affinity membrane receptor for melatonin from the rat, sheep and human male reproductive system and/or brain have been reported. Specific *in vivo* binding of melatonin to its receptors in rat brain has also been demonstrated and the mammalian hypothalamic receptor was shown to be coupled to inhibition of adenylyl cyclase, decreasing cAMP (15-18). Cyclic AMP has been proposed as the intracellular messenger involved in mammalian sperm motility (10), so melatonin might have an effect on cAMP levels to influence sperm motility as in this experiment.

The stimulatory effect of melatonin on sperm motility as reported in the present study, may be due to the property of melatonin as an efficient free radical scavenger and general antioxidant (19,20). Recent discovery implies that melatonin effects every subcellular compartment in every cell since it is both lipophilic and hydrophilic. These intracellular actions of melatonin, some of which are independent of any receptor interaction and some of which are mediated by nuclear receptors, have become the focus of much of the current investigation. As an antioxidant, melatonin has been shown *in vitro* to be a highly efficient scavenger of the very reactive and toxic hydroxyl radical. Likewise, melatonin was found to also scavenge the peroxyl radical which is generated during lipid peroxidation; in this regard it was roughly twice as effective as vitamin E (19,20). Finally, membrane lipid peroxidation, induced either *in vivo* or *in vitro* by any of several means, all of which involve free radicals, is drastically attenuated in the presence of melatonin (19,20). It was demonstrated that centrifugal pelleting of sperm from human ejacu-

Figure 2: Mean (±SE) % progressive motility of normal spermatozoa, when incubated with and without melatonin (*; differences are significant when compared to control samples incubated without melatonin).



lates caused the production of reactive oxygen species within the pellet, which induced irreversible damage to the spermatozoa and defective sperm function at the cellular level (21,22). With this knowledge in mind, it may be anticipated that melatonin's effect as an efficient antioxidant has led to its stimulatory action on sperm motility as demonstrated in the present study, by scavenging reactive oxygen species within the pellet produced by centrifugation. The discovery and cloning of a membrane melatonin receptor on neurosecretory cells in the hypothalamus and on hormone secreting cells of the anterior pituitary gland, and the evidence of melatonin-binding sites in spermatozoa, stimulated a great deal of investigation which has failed to prove the involvement of these receptors in the processes by which melatonin influences reproductive physiology. The recent identification of nuclear melatonin receptors as well as the nonreceptor-mediated actions of the indole are currently being examined as to their association with reproductive function (19,23).

Besides the bulky evidence of melatonin's antireproductive effects on the mammalian reproductive physiology, it also appears from the literature that this indole is capable of modifying mammalian sexual development in a positive manner. Moreno *et al.* suggested that neonatal melatonin administration induced an earlier sexual maturation in male rats, possibly related to prolactin, luteinizing hormone, monoamine oxidase and phenylethanolamine-N-methyl transferase increases (24). Prolactin is gonadotropic at least in some species. Although its role in male reproduction is uncertain; it can bind to prostate and testis, and may augment LH binding to the Leydig cell to facilitate the action of LH on the testis (25). Normally high intratesticular testosterone level is needed for spermatogenesis, and melatonin, by leading to an increase in prolactin and therefore causing gonadotropic action, may enhance spermatogenesis. What is more, Bhagat *et al.* (26) obtained melatonin and steroid-free fractions from ovine pineals, in an attempt to define the antigonadotrophic activity associated with the protein/peptide extracts. In this study, determination of an inhibin-like

antigonadotrophic activity in the ovine pineal strongly supports the view that this peptide may be the major player in what has so far been referred to as antigonadotrophic activity.

Evaluation of our findings with those in the literature suggests that the role of melatonin in human reproductive physiology remains obscure. The stimulatory effect of melatonin that we have determined on human sperm motility may be explained by the antioxidant action of this indole, but in addition melatonin and other indoleamines possibly have direct effects on sperm motility through its membrane and/or nuclear receptors. The results indicate that melatonin might play a role in sperm motility. Further studies are planned to evaluate the effects of melatonin on the sperm flagellum and microtubules at the ultrastructural level, and to investigate its efficiency in scavenging free radicals within the sperm pellet which might contribute to stimulate sperm motility.

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