

SHORT COMMUNICATION

MODULATION OF SERUM CONCENTRATIONS OF MELATONIN
BY CARBAMAZEPINE AND VALPROATE

MADHUR GUPTA, KAMLESH KOHLI* AND Y. K. GUPTA**

*Neuropharmacology Lab., Department of Pharmacology,
All India Institute of Medical Sciences,
New Delhi - 110 029*

and

**Department of Pharmacology,
Lady Hardinge Medical College,
New Delhi - 100 001*

(Received on July 2, 2005)

Abstract : Evidence has accumulated about the involvement of reactive oxygen species (ROS) in epilepsy. The neuromodulator melatonin has been shown to reduce oxidative stress in various animal models due to its free radical scavenging properties. The present study investigated whether carbamazepine and valproate alter serum concentrations of melatonin. Epileptic children were randomly assigned to receive carbamazepine/valproate monotherapy till 22 patients were recruited in the study. At the tenth day, in the evening, samples were drawn for baseline endogenous melatonin estimation. The patients were then administered exogenous melatonin, and repeat samples were drawn after 30 minutes. Serum levels of melatonin were estimated using Melatonin ELISA kits. The median levels of melatonin were 165.0 pg/ml (Range 50.0-350.0) in CBZ+MEL group and 78.0 pg/ml (Range 13.0-260.0) in the VPA+MEL group. The observed difference in melatonin levels could be attributed to the difference in antiepileptic drugs, additive increase in reactive oxygen species due to disease combined with carbamazepine, or possibly to a difference in melatonin kinetics in conditions of oxidative stress.

Key words : epilepsy children valproate
 carbamazepine melatonin oxidative stress

INTRODUCTION

Evidence has accumulated about the involvement of reactive oxygen species (ROS)

in epilepsy (1). The free radicals generated cause a cascade of neurochemical events leading to neurodegeneration. Ironically, though some antiepileptic drugs inhibit free

**Corresponding Author : E-mail : yk.ykgupta@gmail.com; Fax : +91-11-26864789

radical generation, some like carbamazepine (CBZ) are also known to generate free radicals (2). However, the data about free radical generation by sodium valproate (VPA) is not so conclusive (3).

The neuromodulator melatonin has been shown to reduce oxidative stress in various animal models, because of its antioxidant as well as free radical scavenging properties (4), and to antagonize a mutagenic effect of CBZ in some systems (5). Melatonin also exhibits anticonvulsant activity in different seizure models (6) and there has been at least one clinical report suggesting its potential efficacy in a child with severe myoclonic epilepsy (7).

Since the different antiepileptics modulate melatonin not in a similar manner, it is possible that similar dose of add-on melatonin may qualitatively behave differently because of complex interaction with antiepileptics drugs. The objective of the present study was to investigate whether carbamazepine and valproate alter serum concentrations of melatonin.

PATIENTS AND METHODS

Epileptic children, aged between 3–12 years of either sex, who presented to the seizure clinic at the Kalawati Saran Children's Hospital, Lady Hardinge Medical College, New Delhi between April, 2002, and February, 2003, were enrolled. All patients were assessed and screened for inclusion/exclusion criteria (n=45). The institutional scientific and ethical committee approved the study protocol, and the written informed consent was obtained from the accompanying parent/relative. Patients who were on CBZ

monotherapy since last 6–9 months, had a confirmed diagnosis of epilepsy limited to partial or generalized seizures as classified according to the ICES, and were seizure free at least for the last six months were included. Patients with a history of psychiatric or progressive neurological disorder or a chronic hematological, cardiac, hepatic, renal or thyroid disorder were excluded. The patients were randomly assigned to two groups, one group to receive carbamazepine and the other to receive valproate monotherapy till 22 patients were included in the study. On the tenth day of beginning the AED (CBZ/VPA) patients were admitted in the pediatrics ward, subject to their giving the written informed consent for participation. At 7.00 pm, a blood sample was collected for baseline estimation of endogenous melatonin. The patients were then administered oral melatonin tablets as per their age and weight. Melatonin tablets of 3 mg strength (Aristo Pharmaceuticals Ltd, Mumbai, India) were used. The dose of melatonin was 6 mg (2 tablets) for children less than 9 years/ weighing less than 30 kg, and 9 mg (3 tablets) for children more than 9 years/ weighing more than 30 kg. The doses of melatonin administered were based on those used by Jan and Donell (8). After half an hour, at 7.30 pm, another blood sample was collected for the estimation of serum levels of exogenous melatonin administered. Blood was then centrifuged at 3500 rpm to separate out the serum, and stored in eppendorf tubes at -85°C in the deep freezer till estimation. Serum levels of melatonin on exogenous administration of melatonin were estimated using Melatonin ELISA kit (IBL Immunobiological Laboratories, Hamburg Germany) with the sensitivity of 3 pg/ml in the department of Pharmacology

and Microbiology, Lady Hardinge Medical College.

STATISTICAL ANALYSIS

Descriptive statistics were calculated for all the outcome variables, and expressed as median (range). The Wilcoxon signed rank test was used to compare pre and post treatment values within each group. The Chi Square test was used to compare the categorical variables with two different groups. There was no power calculation done because this was an explorative study. P value <0.05 was considered as significant. All the data were analyzed using STATA 7.0 (intercooled version).

RESULTS

Estimation of serum levels of Melatonin in the CBZ+MEL & VPA+MEL group by ELISA

The pretreatment median serum concentration of melatonin in the Carbamazepine + Melatonin group was 1.5 pg/ml (range 0.8–15.0 pg/ml) as compared to 1.3 pg/ml in the Valproate + Melatonin group. There was no significant difference in the levels of melatonin in the two groups pretreatment ($p=0.68$). On exogenous administration of melatonin in both the groups, there was a remarkable increase in levels of melatonin in the blood (sample taken after 30 minutes of melatonin administration), in both the treatment groups. The median levels of melatonin were 165.0 pg/ml (Range 50.0–350.0) in CBZ+MEL group and 78.0 pg/ml (Range 13.0–260.0) in the VPA+MEL group. The levels of melatonin in the carbamazepine group were

almost twice as compared to the valproate group. Though, there was a marked increase in levels of melatonin post treatment, due to wide variation in data, the difference in the percentage increase in the two groups was not statistically significant ($P=0.75$) (Table I).

TABLE I: Effect of melatonin serum levels of CBZ/VPA.

<i>Study variables</i>	<i>Carbamazepine+ Melatonin Median (Range)</i>	<i>Valproate+ Melatonin Median (Range)</i>	<i>P value</i>
Serum concentrations of Melatonin (pg/ml) (ELISA)			
Pre treatment	1.5 (0.8–15.0)	1.3 (1.0–1.7)	0.68
Post treatment	165.0 (50.0–350.0)	78.0 (13.0–260.0)	0.31
% Increase	8566.7 (233.0–43650.0)	4775.0 (1200.0–19900.0)	0.75

DISCUSSION

The disease process of epilepsy as well as the antiepileptic drugs lead to generation of free radicals, which may be responsible in the modulation of melatonin levels. There is by far no direct evidence to suggest the change in melatonin level by carbamazepine or valproate in a clinical setting. No other factor attributable to the pathophysiological/disease condition was observed, which could be considered responsible for the observed difference in melatonin levels in the valproate and carbamazepine groups. It could be postulated to be due the difference in the antiepileptic drugs. The reason why a high level of melatonin was found in the carbamazepine group is difficult to

explain with this study. The additive increase in reactive oxygen species because of the disease process combined with carbamazepine may be hypothesized for the net increase in ROS (though not measured in the present study). One possible explanation for our findings is that the increased ROS accumulation (2) could also be responsible to either produce more melatonin or conserve the available melatonin so that higher melatonin levels are made available to meet the increased

antioxidant demand due to a higher free radical load. It is possible that in conditions of increased oxidative stress, the melatonin kinetics may be different, and there may be a downregulation of the enzymes responsible for degradation of melatonin. In the nucleus, melatonin binds to retinoid Z receptor (RZR), whose response elements have been found in some important genes related to oxidative stress (9). There is a need for further studies to explore the interaction and modulation of melatonin with antiepileptic drugs.

REFERENCES

1. Torbati D, Church DF, Keller JM, et al. Free radical generation in the brain precedes hyperbaric oxygen induced convulsions. *Free Radical Biol Med* 1992; 13: 101–106.
2. Reiter RJ, Tan DX, Sainz RM, et al. Melatonin: reducing the toxicity and increasing the efficacy of drugs. *J Pharm and Pharmacol* 2002; 54: 1299–1321.
3. Wang JF, Azzam JE, Young LT. Valproate inhibits oxidative damage to lipid and protein in primary cultured rat cerebrocortical cells. *Neuroscience*. 2003; 116(2): 485–489.
4. Allegra M, Reiter RJ, Tan DX, et al. The chemistry of melatonin's interaction with reactive species. *J Pineal Res* 2003; 34: 1–10.
5. Awara WM, El-Gohary M, El-Nabi SH, et al. *In-vivo* and *In-vitro* evaluation of the mutagenic potential of carbamazepine: does melatonin have anti-mutagenic property? *Toxicology* 1998; 125: 45–52.
6. Srivastava AK, Gupta SK, Jain S, Gupta YK. Effect of melatonin and phenytoin on an intracortical ferric chloride model of post-traumatic seizures in rats. *Methods Find Exp Clin Pharmacol* 2002; 24: 145–149.
7. Molina-Carballo A, Munoz-Hoyos R, Reiter RJ, et al. Utility of high dose of melatonin as adjunctive anticonvulsant therapy in a child with severe myoclonic epilepsy: two years experience. *J Pineal Res* 1997; 23: 97–105.
8. Jan JE, O'Donnell ME: Use of melatonin in the treatment of paediatric sleep disorders. *J Pineal Res* 1996; 21: 193–199.
9. Rodriguez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, Reiter RJ. Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res* 2004; 36: 1–9.