ORIGINAL ARTICLE

Agomelatine's effect on human genetic material: *in vitro* study

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ABSTRACT: ***Introduction:*** Agomelatine is a prescription drug approved for the treatment of major depressive disorder. It is a melatonergic agonist and a 5-HT2C antagonist. The cytogenetic behavior of agomelatine has not been studied. The aim of the present study is the investigation of the *in vitro* effect of agomelatine on human DNA, by estimating sensitive cytogenetic indices. ***Methods:*** SCEs (Sister Chromatid Exchanges) are considered as one of the most sensitive markers of genotoxicity, PRI (Proliferation Rate Index) is one of the most reliable markers of cytostatic activity, whereas MI (Mitotic Index) shows precisely the ability of the cell to proliferate. We have investigated the effect of five agomelatine solutions on SCEs, PRI and MI of human cultured lymphocytes stained with the Fluorescence plus Giemsa method and estimated with the optical microscope. ***Results:*** Analysis of the results has revealed statistically significant ($p<0.001$) dose-dependent increase of SCE frequencies and significant reduction of PRI and MI values on lymphocyte cultures treated with agomelatine. Furthermore, a correlation was observed between a) the magnitude of the SCE induction and the PRI alterations, b) the magnitude of the MI alterations and the SCE induction and c) the magnitude of PRI alterations and MI alterations. ***Conclusions:*** Agomelatine at therapeutic doses exhibited dose-dependent cytogenetic activity *in vitro*. This may provide additional information about the mechanism of action of the drug. Considering that the use of agomelatine has rapidly increased, further studies in other cell lines and in vivo experimental settings are needed in order to evaluate its effect on human genetic material.

Keywords: Agomelatine, Cytogenetic Activity, Sister Chromatid Exchanges, Proliferation Rate Index, Mitotic Index.

INTRODUCTION

Agomelatine is a melatonergic agonist, which exerts its effect through Melatonin 1 (MT1) and Melatonin 2 (MT2) receptors, and it is also a selective serotonin antagonist for 5-HT2C receptors. MT1 receptors are localized mainly in the suprachiasmatic nuclei (SCN) and the pars tuberalis whereas MT2 receptors are expressed in the SCN and retina[1,2]. Furthermore, agomelatine increases noradrenaline and dopamine release specifically in the frontal cortex, while it has no influence on the extracellular levels of serotonin and it appears to improve sleep quality, with no reported daytime drowsiness[1,3,4].

Agomelatine (beta-methyl-6-chloromelatonin) is structurally homologous to melatonin, a neurohormone involved in the regulation of circadian rhythms, with a wide-spectrum antioxidant activity[5]. Melatonin is thought to be the most effective lipophilic antioxidant that can easily cross cell membranes[6] and the blood–brain barrier[7] (Figure 1).

Agomelatine is absorbed rapidly and satisfactorily after oral administration but its bioavailability is less than 5% because of the high first pass metabolism. This drug is biotransformed mainly by cytochrome P450 1A2 (CYP1A2). It is indicated for Major Depressive Disorder (MDD) in adults due to its antidepressant effects. It has also positive effects on sleep architecture in these patients and it is the first antidepressant drug which does not block the reuptake of monoamines[2,8]. In addition, agomelatine resynchronizes circadian

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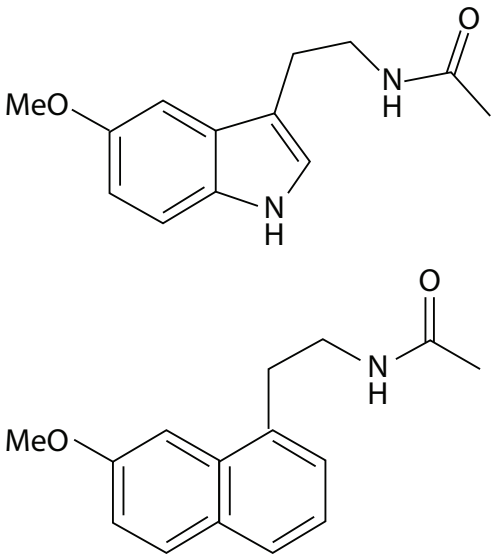

Figure 1.

rhythms and it has recently been proposed as a treatment for anxiety disorders such as panic disorder (PD) and social anxiety disorder (SAD) due to its anxiolytic 5-HT2C blokable action. Because of this, agomelatine's role has been investigated in pharmacotherapy of obsessive-compulsive disorders (OCDs)[3,9,10]. Furthermore, agomelatine seems to be an effective drug for acute depression in Bipolar Disorder (BD) type II as the circadian rhythm hypothesis of BD suggests a role for melatonin in regulating mood[11,12].

Finally, agomelatine causes fewer sexual side effects and discontinuation effects than other antidepressant medications[8,13].

Though literature data about clinical efficacy, safety and adverse effects of agomelatine are satisfactory, its behavior at molecular level has not been investigated at all.

The aim of the present study is to investigate the *in vitro* cytogenetic effect of agomelatine on human cultured T-lymphocytes by estimating sensitive indices: SCEs, PRI and MI. SCEs has been identified as one of the most sensitive indices among biomarkers of genotoxicity, PRI and MI have been used as sensitive indicators for the evaluation of the cytostatic activity of various environmental hazards or therapeutic agents[14].

MATERIALS AND METHODS

***In vitro* SCE, PRI and MI assays.** Blood given by four healthy donors 19-21 years old, who were non-smokers, not receiving any drugs, not consuming considerable quantities of alcohol and not having suffered any kind of infection for the last 15 days, was used to be culture lymphocytes for the SCE, PRI and MI assays.

Human T-lymphocyte cultures were prepared by adding in each of six sterile universal tubes containing 5 ml chromosome medium (RPMI-1640, Biochrome, supplemented with 20% fetal calf serum, 0,63% L-glutamine, 0,63% penicillin/streptomycin and 2% phytohaemagglutinin) at the beginning of the culture life, the following:

- 11-12 drops of human peripheral heparinized whole blood
- 0.1ml of 5-bromodeoxyuridine (BrdU) water solution (500 μg/ml),

in addition, in experimental tubes:

- 0,1 ml agomelatine solutions (A=2.5μg/ml, B=5μg/ml, C=10μg/ml, D=15μg/ml, E=20μg/ml final concentrations per corresponding culture) and

in the control tube:

- 0.1 ml of agomelatine solvent: double distilled water: ethanol, 9:1.

The agomelatine concentrations B and C were equivalent to the most common used therapeutic dosages per os (25-50 mg/day). The cultures were incubated at 37° C for 72 hours in the dark to minimize photolysis of 5-BrdU. Colchicine (0.3μg/ml water solution) was added 2h before the collection of the cultures. The cells were then collected by centrifugation and exposed to 0.075M KCl for 10 minutes. The hypotonic solution spreads the chromosomes and hemolyses the red blood cells. The pellet was fixed three times with methanol: acetic acid (3:1). Drops of concentrated suspension of cells were placed on microslides that allow to air dry. For SCEs, PRI and MI analysis, the slides were stained by a modification of the fluorescence plus Giemsa procedure to obtain harlequin chromosomes[15].

Statistical Analysis. For SCE estimation, 30 suitably spread second division cells from each culture were blindly scored. For PRI calculation 100 cells in the first, second, third and higher divisions from each culture were blindly scored. PRI=M1+2M2+3M3+.../100, where M1, M2 and M3+... are the percent values of cells in the first, second, third and higher divisions, respectively. For MI analysis, all cell divisions present in an optical field of 1000 nuclei were scored. MI=number of cells in mitosis/total number of nuclei (1000). For the statistical evaluation of the experimental data, Student's t-test was performed to determine whether any SCE values differed significantly from the controls and

the χ2-test was used for PRI and MI comparisons. Simple linear correlation between SCEs and PRI frequencies, SCEs and MI frequencies and PRI and MI frequencies were also calculated using Pearson's product moment correlation coefficient.

RESULTS

Table I illustrates the effect of agomelatine on SCE frequency. Cultured T lymphocyte SCE frequency is presented as dose-dependent increase after the effect of agomelatine solutions: B=5μg/ml, C=10μg/ml, D=15μg/ml and E=20μg/ml (final concentrations per culture). This increase is statistically significant (P<0,001) and indeed the concentration of 10μg/ml duplicates the SCE rate in cultured lymphocytes of all blood donors, though a small decrease (not statistically significant) in SCE rate is observed at concentration of 2.5μg/ml in all lymphocyte cultures.

Table I. Effect of Agomelatine on SCE frequency in human lymphocyte cultures from four healthy donors

Dosage (μg/ml)	SCEs /cell 1st donor	2nd donor	3rd donor	4th donor
Control (H_2O)	5,28	5,12	6,01	7,1
2,5	5,1	4,8	5,9	6,9
5	7,9*	7,43*	9,07*	9,87*
10	10,16*	9,93*	11,31*	12,22*
15	12,71*	11,49*	13,68*	13,97*
20	15,1*	14,82*	14,92*	15,6*

***Statistically significant (p<0,001) increase over the corresponding control (t-test)**

SCEs have been correlated with corresponding PRI values (r= -0.971, p<0.001).

The Pearson product moment correlation coefficient r was applied for calculating the correlation between SCEs and PRIs. A criterion for testing whether r differs significantly from zero was used whose sampling distribution is Students test with n-2 degrees of freedom.

Table II illustrates the effect of agomelatine on PRI. Agomelatine solutions of 2.5μg/ml, 5μg/ml,10μg/ml and 15μg/ml induce not statistically significant decrease, though the solution of 20μg/ml causes a remarkable decrease on PRI values of cultured T lymphocytes, statistically significant (P<0,001).

Table III presents the effect of agomelatine on MI values. Agomelatine solutions cause a decrease of MI on lymphocytes. Specifically the solutions of 5μg/ml, 10μg/ml, 15μg/ml and 20μg/ml induce statistically significant decrease (P<0,001). Furthermore, a correlation was observed (P<0.001) between:

1. the magnitude of the SCE induction and the MI alterations
2. the MI alterations and PRI alterations and
3. the magnitude of the SCE induction and the PRI alterations

Table II. Effect of Agomelatine on PRI frequency in human lymphocyte cultures from four healthy donors

Dosage (μg/ml)	SCEs /cell 1st donor	2nd donor	3rd donor	4th donor
Control (H_2O)	2,41	2,45	2,4	2,52
2,5	2,34	2,37	2,33	2,43
5	2,25	2,29	2,26	2,36
10	2,29	2,33	2,31	2,40
15	2,33	2,36	2,32	2,42
20	2,14**	2,17**	2,21**	2,23**

****Statistically significant (p<0.001) decrease over the corresponding control (χ^2 test)**

Table III. Effect of Agomelatine on MI frequency in human lymphocyte cultures from four healthy donors

Dosage (μg/ml)	SCEs /cell 1st donor	2nd donor	3rd donor	4th donor
Control (H_2O)	38	40	39	43
2,5	36	37	36	40
5	30***	29***	29***	35***
10	31***	32***	30***	37***
15	34	33***	33***	38
20	25***	26***	26***	30***

*****Statistically significant (p<0.001) decrease over the corresponding control (χ^2 test)**

DISCUSSION

Depression is one of the most significant causes of disability worldwide. Despite the variety of effective treatment strategies available, almost one third of the patients fail to respond to antidepressant therapies[1]. There is a need for more effective and better tolerated antidepressants to combat the depression characteristics (behavioral, cognitive and emotional). Understanding the pathophysiology of depression and finding new treatment strategies is of great importance.

Agomelatine is a novel drug recently added to the list of the antidepressants available. The specificity of its mechanism of action, as a potent agonist of melatonin MT1 and MT2 receptors as well as an antagonist of serotonin 5-HT(2C) receptors, may provide a useful,

alternative pharmacological strategy to existing antidepressant drugs[2,3,4].

The present research investigates the cytogenetic behavior of agomelatine on healthy human T lymphocyte cultures estimating the most reliable indices of cytogenetic damage: SCEs, PRI and MI. In addition, this method also has the advantage of testing the indices mentioned above in human CD4 and CD8 T lymphocytes. Recent data from laboratory animal studies show that T lymphocytes may have a protective role against depression by producing IL-4. This production leads to the increase of Brain Derivative Neurotrophic Factor (BDNF) levels by astrocytes and to the conversion of meningeal macrophages to M2 phenotype. Both CD4 and CD8 T lymphocytes also express melatonin receptors[16,17,18,19].

The SCEs significant induction (Figure 2) of cultured lymphocytes from all four blood donors after treatment with therapeutic doses of agomelatine may indicate an insufficiency in DNA damage repairing. High SCEs values could also demonstrate a considerable DNA damage which could not be repaired before T cells reach S phase *in vitro*[20,21]. This means that agomelatine induces DNA damage at therapeutic doses *in vitro* or that agomelatine affects cell repair system, which is notable to repair the damage caused[22]. This should be further investigated. The possible alterations in immune response of T lymphocytes and cytokine secretion caused by DNA damage should also be investigated. In Table I it is also shown that subtherapeutic dose of agomelatine (2.5μg/ml) causes a reduction of SCE frequency. Although the decrease is not statistically significant, it is an interesting finding in cultured lymphocytes of all donors, considering the protective role of T lymphocytes in depression[16]. Agomelatine in smaller doses may have a protective role to DNA molecule possibly due to the reinforcement of repair mechanisms.

As recently the interest of scientists about the role of inflammation in depression is increased, cultured lymphocyte cytogenetic response after agomelatine treatment provides us with further information for the mechanism of action of agomelatine in the inflammatory pathway of depression.

According to Table II therapeutic doses of agomelatine (Figure 3) do not affect the proliferation rate (PRI) and only the higher concentration of 20μg/ml induces a remarkable decrease on PRI values of cultured T lymphocytes, statistically significant ($P<0,001$).

On the contrary, the statistically significant induction of lymphocyte MI values (Figure 4) after the effect of therapeutic doses shows that agomelatine has a cytostatic behavior *in vitro*, which needs further investigation.

This preliminary study shows that agomelatine has a very interesting cytogenetic behavior and it also provides information about the unknown inflammatory

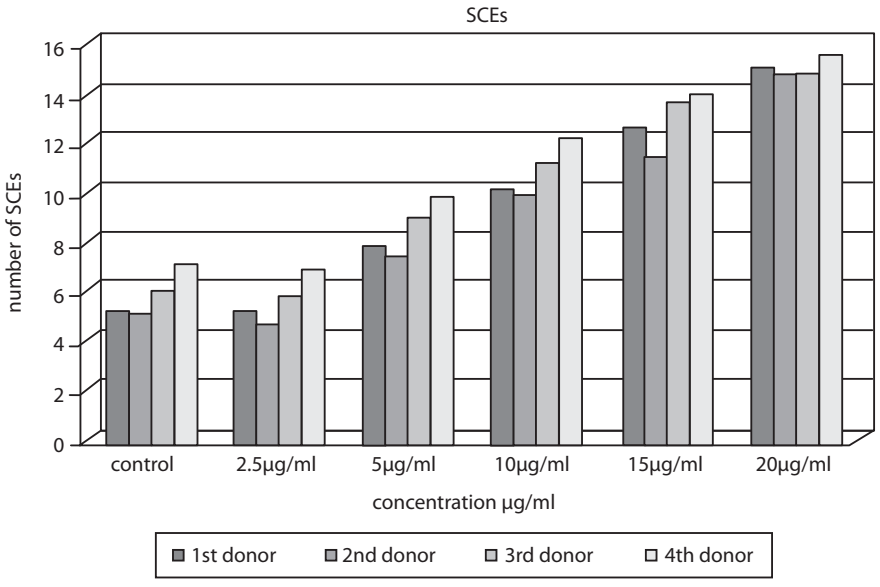

Figure 2. Effect of Agomelatine on SCE frequency in human lymphocyte cultures from four healthy donors.

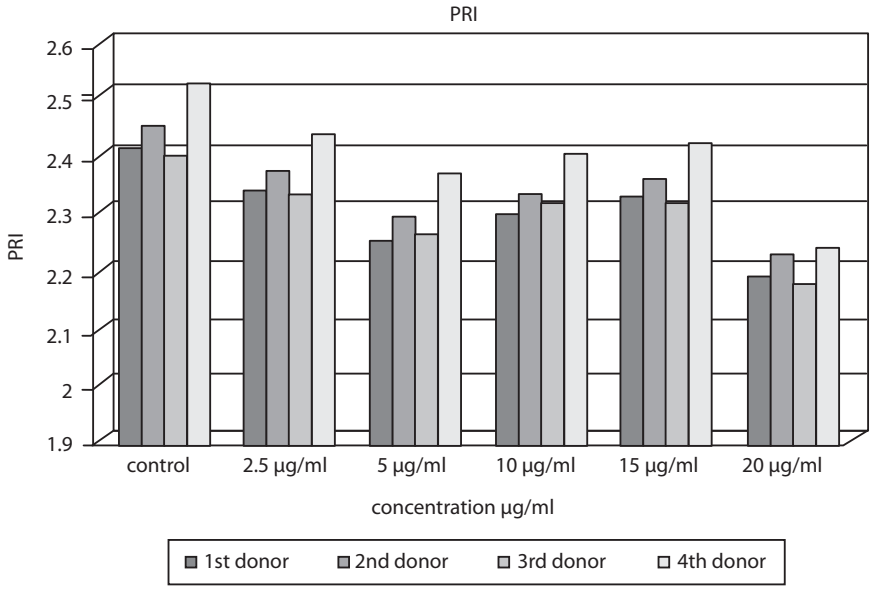

Figure 3. Effect of Agomelatine on PRI frequency in human lymphocyte cultures from four healthy donors.

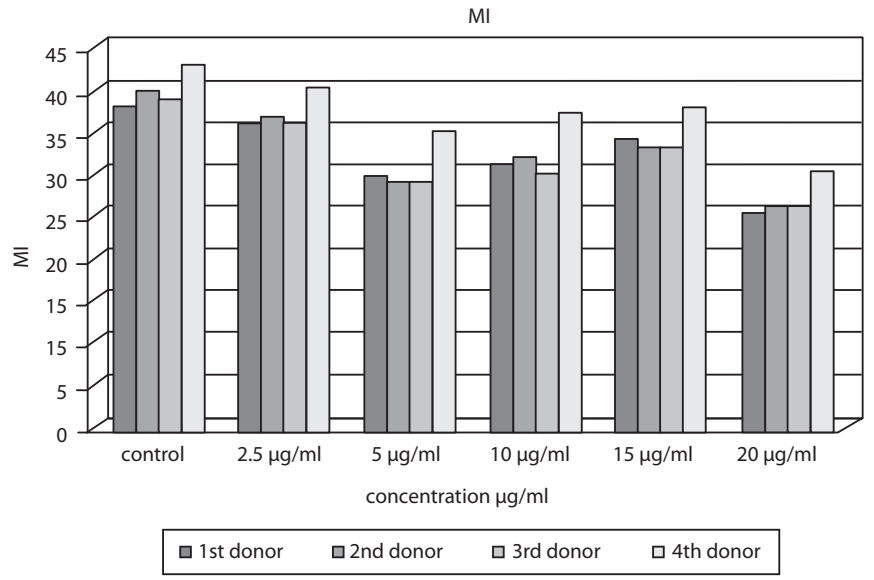

Figure 4. Effect of Agomelatine on MI frequency in human lymphocyte cultures from four healthy donors.

path of depression. Our next step is the investigation of cytogenetic behavior of agomelatine at a larger amount of donors, at different cell lines (B lymphocytes and macrophages), in vivo experiments and DNA repair enzymes (DNA ligase, topoisomerases) measurements.

Disclosure Statement

No competing financial interests exist.

Επίδραση της Αγομελατίνης στο Γενετικό Υλικό του Ανθρώπου: *In vitro* μελέτη

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Εισαγωγή: Η αγομελατίνη αποτελεί ένα εγκεκριμένο φάρμακο για τη θεραπεία της μείζονος καταθλιπτικής διαταραχής. Παρουσιάζει δράση μελατονινεργικού αγωνιστή και ανταγωνιστή των 5-HT2C υποδοχέων της σεροτονίνης. Σκοπός της παρούσας έρευνας είναι η μελέτη της επίδρασης της αγομελατίνης στο ανθρώπινο DNA *in vitro* με τον υπολογισμό ευαίσθητων κυτταρογενετικών δεικτών.

Υλικό και Μέθοδος: Οι χρωματιδιακές ανταλλαγές (Sister Chromatid Exchanges, SCEs) θεωρούνται ένας από τους πιο ευαίσθητους δείκτες γονοτοξικότητας. Ο δείκτης ρυθμού πολλαπλασιασμού (Proliferation Rate Index, PRI) είναι ένας από τους πιο αξιόπιστους δείκτες κυτταροστατικότητας, ενώ ο μιτωτικός δείκτης (Mitotic Index, MI) δείχνει με ακρίβεια την ικανότητα του κυττάρου για πολλαπλασιασμό.

Αρχικά παρασκευάστηκαν διαλύματα αγομελατίνης πέντε διαφορετικών συγκεντρώσεων (A=2.5μg/ml, B=5μg/ml, C=10μg/ml, D=15μg/ml και E=20μg/ml). Οι συγκεντρώσεις B και C είναι οι πιο συχνά χρησιμοποιούμενες στην κλινική πράξη. Τα διαλύματα προστέθηκαν σε καλλιέργειες λεμφοκυττάρων από περιφερικό αίμα τεσσάρων νεαρών υγειών αιμοδοτών. Μετά από 72 ώρες επώασης, με την κατάλληλη τεχνική τα καλλιεργημένα λεμφοκύτταρα επιστρώθηκαν σε αντικειμενοφόρους πλάκες, χρωματίστηκαν με την μέθοδο Fluorescence plus Giemsa και οι προαναφερθέντες δείκτες υπολογίστηκαν με οπτικό μικροσκόπιο.

Αποτελέσματα: Η ανάλυση των αποτελεσμάτων αποκάλυψε στατιστικά σημαντική αύξηση των χρωματιδιακών ανταλλαγών και μείωση τόσο του δείκτη ρυθμού πολλαπλασιασμού όσο και του μιτωτικού δείκτη . Επιπρόσθετα, προέκυψε συσχέτιση μεταξύ α) της αύξησης των SCEs και των μεταβολών του MI και του PRI και β) των μεταβολών MI-PRI.

Συμπεράσματα: Η αγομελατίνη σε θεραπευτικές δόσεις προκάλεσε δοσοεξαρτώμενες μεταβολές στους υπό μελέτη κυτταρογενετικούς δείκτες. Το παραπάνω ενδέχεται να καταδεικνύει στοιχεία για το μηχανισμό δράσης του φαρμάκου. Λαμβάνοντας υπόψη την αυξανόμενη χρήση της αγομελατίνης επιβάλλεται περεταίρω μελέτη της κυτταρογενετικής της δράσης σε περισσότερους αιμοδότες καθώς και σε άλλες κυτταρικές σειρές.

Λέξεις-Κλειδιά: Αγομελατίνη, Γονοτοξικότητα, Χρωματιδιακές ανταλλαγές, Δείκτης ρυθμού πολλαπλασιασμού, Μιτωτικός δείκτης

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