ORIGINAL ARTICLE

Melatonin enhances the *in vitro* action of cytochalasin B on globular resistance and osmotic fragility of erythrocytes: a preliminary study

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Abstract

BACKGROUND: Some researches have shown that melatonin (MLT) has effects on the erythrocyte deformability and on the osmotic fragility which, currently, seem to be heterogeneous and fragmentary.
OBJECTIVE: This work wished to evaluate *in vitro* the possible modifying action of MLT at pharmaceutical doses on the osmotic fragility of rat blood samples treated with cytochalasin B (CB). The variation of the lactate dehydrogenase (LDH) levels have been measured because LDH is an enzyme which is considered an important marker of hemolysis. Working in a strongly reducing environment has been necessary in order to avoid possible antioxidant actions of MLT, measuring the plasma levels of malondialdehyde and total phospholipids in order to highlight possible MLT actions which are not related to its recognized antioxidant properties.
RESULTS: The data show a possible MLT action which strengthens the CB action on the osmotic fragility and on the membrane deformability of the erythrocytes.
CONCLUSIONS: Under the adopted working conditions, a direct relation of MLT with the biochemical dynamics of the cytoskeleton-mediated processes can be suggested. Further studies will be needed to clarify the mechanisms and the extent of the observed phenomena.

INTRODUCTION

N-acetyl-5-methoxytryptamine or melatonin (MLT) besides being the main secretion of the pineal gland or epiphysis, also seems to be an autocrine/paracrine factor in the immuno-hematopoietic processes (Di Bella *et al.* 1969; 1976; 1979a,b; Rossi *et al.* 1976; Di Bella & Rossi 1980; Rossi & Di Bella 1988; Conti *et al.* 2000; Pacini & Borziani 2009). Such a substance is synthesized by the primary lymphoid and hematopoietic organs, namely the red bone marrow, the thymus and in many cells of such a system, amongst which the T lymphocytes, the mononuclear phagocytes and the platelets (Launay *et al.* 1982; Champier *et al.* 1997; Tan *et al.* 1999; Carrillo-Vico *et al.* 2004; Morera & Abreu 2005; Naranjo *et al.* 2007). Furthermore, also the red blood cells are able to convert serotonin into MLT *in vitro* (Rosengarten *et al.* 1972).

Many cells of the immuno-hematopoietic system possess and express receptors for MLT, both for membrane belonging to the family of G-protein coupled receptors (GPCR), in particular, belonging to Gi and Gq subgroups, and for specific nuclear receptors in lymphocytes and other cells of the immuno-hematopoietic system. This fully explains the autocrine/paracrine action as well as its systemic and endocrine action related with darkness (Vacas *et al.* 1992; Pozo *et al.* 1997; Drazen *et al.* 2001; Yau *et al.* 2002; Carrillo-Vico *et al.* 2005; Szczepanik 2007; Regodón *et al.* 2009). Moreover, MLT also has a direct action to inhibit the outwards currents in the megakaryocytes and a similar action in the lymphocytes where it directly binds the Kv1.3 potassium channels (Varga *et al.* 2001; Di Bella *et al.* 2002).

Some work has demonstrated that MLT has a considerable cytoprotective effect on the lipid peroxidation of the erythrocyte membrane (Tesoriere *et al.* 1999; Aydogan *et al.* 2004; Yerer *et al.* 2004). However, according to physiological studies, it would lead to a clear reduction of the erythrocyte deformability: this applies to both researches *in vitro* and *in vivo* with rats exposed to an alteration of the light/dark cycle. In particular, according to Yerer & Aydogan (2006), the constant exposition to the dark in the 24 hours diminishes the erythrocyte deformability in the rat.

However, by analysing the entire literature data, it can be supposed that the actions of MLT on the erythrocyte deformability and on the osmotic globular fragility are diverse and heterogeneous. Particularly, these actions are conditional on the adopted dose and the oxidative states of the hemoglobin and the erythrocyte membrane. More precisely, under high oxidative stress conditions due to cumene hydroperoxide and sodium nitroprusside, MLT shows to be able to offer a good protection against lipid peroxidation, leading to a reduction of malondialdehyde (MDA) formation and to an increase of the deformability and osmotic resistance. Such actions seem to show only at high doses (10 μM–1 mM) and, if the protein oxidation is involved, and in particular the hemoglobin one, these actions of membrane protection remain but a decrease of cell deformability appears, owing to the worsening of oxidative condition in the protein compartment (Tesoriere *et al.* 1999; Aydogan *et al.* 2004; Yerer *et al.* 2004; Dikmenoglu *et al.* 2008). On the whole, the current literature appears to be disconnected and heterogeneous so that it is not possible to express a certain opinion on the possible action of MLT on the erythrocyte deformability and globular resistance.

Cytochalasin B (CB) belongs to the family of compounds whose structure and function are connected to the metabolism of some groups of fungi. CB was isolated for the first time in 1964 as a metabolic product of the Helminthosporium Dematioideum (Aldridge *et al.* 1967). This substance presents particular bioactive properties, amongst which an antiplatelet action (Shepro *et al.* 1970; White *et al.* 1971; Haslam *et al.* 1972) and an action which inhibits the cytodieresis and the cellular movements (Carter 1967; Krishan 1972). Although various members of the family of cytochalasins inhibit the glucose transporter GLUT-1 through a specific binding site located on the erythrocyte membrane, CB is a weak inhibitor of the glucose transport, which is blocked to a minor extent than other cytochalasins (Lin & Spudich 1974; Atlas & Lin 1978; Atlas *et al.* 1980).

Beyond the many actions of cytochalasins, and in particular of cytochalasin D and CB, their main mode of action can be ascribed to the actions they perform on the state of polymerisation of the G-actin in the filaments of F-actin. CB blocks the polymerisation of the G-actin in the F-actin filament, through a mechanism similar to the capping proteins, binding with the barbed ends and destabilising the treadmilling phenomena. This moves the balance between the G-actin and the F-actin towards the former (Lin & Lin 1978; Brenner & Korn 1979; 1980; Flanagan & Lin 1980; Lin *et al.* 1980; Mabuchi 1983). However, some studies have postulated that the destabilisation and depolymerisation of the actin filaments would occur through different mechanisms for CB (Bonder & Mooseker 1986). It is interesting to note how, under physiological conditions, the polymerisation of the actin microfilaments is regulated by some proteins called actin binding proteins (ABP): the so-called capping proteins belong to this complex. They cover the barbed ends blocking the addition and/or the detachment of new subunits. Other proteins such as the profilin binds with the G-actin subunits making it easy the accumulation while the thymosin beta binds with the G-actin, hindering the polymerisation in F-actin, and finally, the severin proteins (gelsolin and fragmin), which also belong to the ABP, cut the filaments of F-actin (Fletcher & Mullins 2010). The interactive action of these proteins demands a cytoplasmic increase of Ca^{2+} ions, which are a pivotal element in the processes of remodelling of the cytoskeleton (CSK)

(Glenney & Weber 1981). In these group of proteins it is possible to identify the protein factor isolated from the platelets by Grumer & Lin (1980) and called cytochalasin-like factor.

CB *in vitro* considerably decreases the osmotic resistance and leads to a clear increase of erythrocyte deformability and of plastic processes responsible for the changes of the erythrocyte shape (Beck *et al.* 1972; Lin & Lin 1978). It is likely to be due to the high affinity bond of the actinic component of the erythrocyte CSK and to the block of action of the GLUT-1. Such actions happen very quickly and seem to be reversible if CB is removed from the medium by microdialysis (Beck *et al.* 1972). In spite of the erythrocyte CSK being small and morphologically different from the one of nucleated cells, it presents submembrane microfilaments very similar to the ones of other cells and it is also sensitive to CB (Lin & Lin 1978; Tsukita *et al.* 1984; Marchesi 1985). In particular, the structure of the erythrocyte CSK has a morphological organisation different from other types of cells (Figure 1): instead of a real tridimensional cytoplasmatic network, the erythrocyte CSK consists of a small submembrane protein network and it also forms together with the cell membrane a system which operates the complex and diverse viscoelastic and deformation processes which are necessary for the erythrocyte function. This allows to the mature red blood cells, whose average life is of approximately 120 days, to move within the vessels whose diameter can be considerably inferior to the erythrocytes' one.

OBJECTIVES

Over the last 15 years some studies have shown the CSK as a possible target of the MLT action (Matsui & Machado-Santelli 1997; Finocchiaro & Glikin 1998; Benítez-King 2006; Benítez-King *et al.* 2009). Furthermore, it has been shown that MLT can find binding sites with high affinity with the F-actin filaments and, very probably, also with the G-actin. Moreover, MLT would also operate a modulating action on the Rho-kinase, so that to reduce the migration of cancer cells, particularly of the MCF-7 cell line. Some of its oncostatic mechanisms would be carried out through the modulation of the Rho-kinase (Ramírez-Rodríguez *et al.* 2007; Ortíz-López *et al.* 2009). Besides, it seems also able to intervene in the regulation of the water metabolism affecting the distribution of aquaporins (vectorial transport of water) in the isolated epithelium of canine nephrons (Ramírez-Rodríguez *et al.* 2003).

Considering the action of MLT on the CSK, and in particular on the actin microfilaments and their

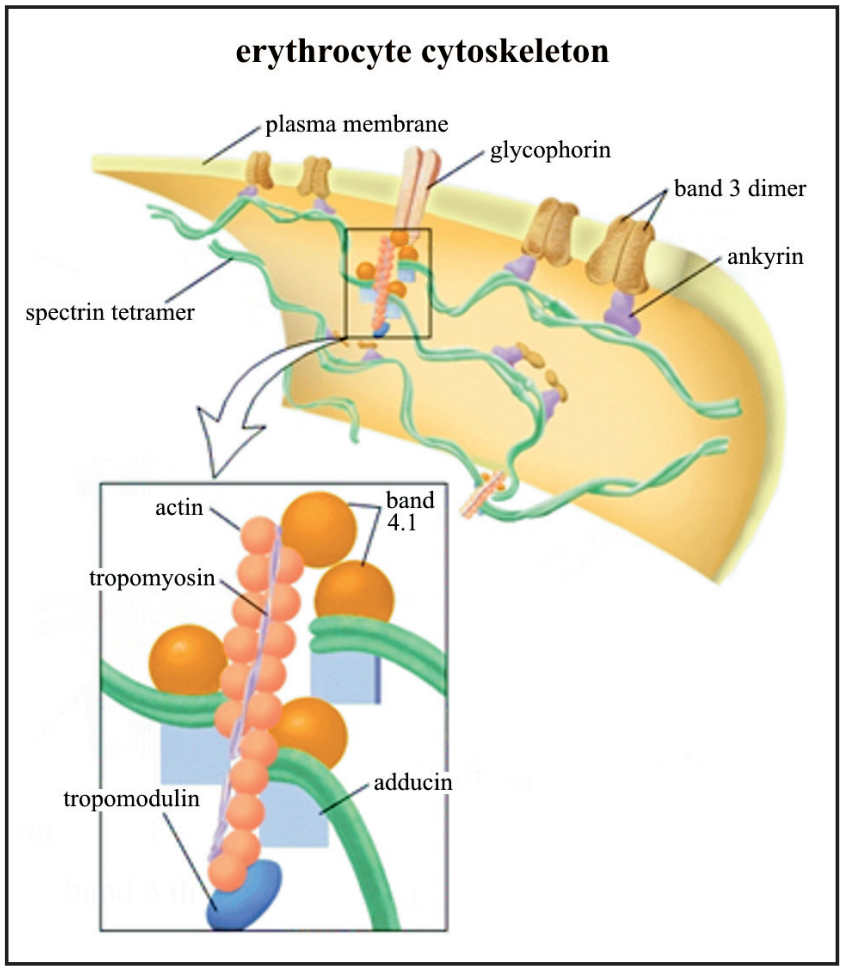

Fig. 1. The erythrocyte CSK consists of a crossed net of spectrin. Every molecule of spectrin is composed by two antiparallel polypeptide chains, with common helix and characterised by weak, non-covalent interactions. Such spectrin filaments are connected together by junctions formed by protein band 4.1, a short actinic filament, tropomyosin and adducin. Therefore, the erythrocyte CSK is in relation with the erythrocyte membrane intrinsic proteins, in particular with the protein band 3 and the glycophorin: such bonds occur with the interposition of the ankyrin for the protein band 3 and of the glycophorin for the protein band 4.1.

dynamics, it is interesting to evaluate if MLT could affect the osmotic resistance of rat erythrocytes which come from blood treated in advance with CB. This is in order to highlight a possible functional relation of agonism/antagonism on the action performed by this substance on the actin microfilaments. If MLT operated an action on the actin component of CSK, this should be shown by modifications in the globular resistance and in the markers of hemolysis.

METHODS

In this study 50–65 days old Wistar rats, weighting 220 ± 16 g were utilized. These rats came from breeders specialised in supplying animals for laboratory research. Once in our laboratory, the rats were reared and treated with the utmost respect for their physiology as specified in the relative guidelines: they were fed ad libitum with a standard balanced diet and kept in an environment with alternation of light/dark of 12 hours at a temperature of 20–22 °C.

MLT (Sigma Aldrich, purity ≥98%) was solubilised in dimethylsulphoxide (DMSO) (Sigma Aldrich, purity ≥99.7%) and brought up to volume with bidistilled water. Solutions were prepared containing 200 mg/100 ml of MLT in water with 2% w/V DMSO.

CB (Sigma Aldrich, purity ≥98%) was solubilised in DMSO and brought up to volume with bidistilled water. Solutions were prepared containing 5 mg/10 ml of CB in water with 2% w/V DMSO.

In order to obtain the data necessary to construct the globular resistance curves, blood samples were taken from a group formed by 10 rats. The animals were anaesthetised with diethyl ether and subsequently about 4 ml of blood were taken from the left ventricle of each rat using microperfusion needles. Then blood was collected into sterile test tubes containing lithium heparin and temporarily maintained at 4 °C.

Each blood sample was then divided into three aliquots of 1 ml: 40 µl of water solution with 2% w/V DMSO have been added to the first and the second. 40 µl of the solution containing MLT have been added to the third one. The three blood aliquots have been placed in a thermostat for 30 minutes at 37 °C.

Afterwards, 100 µl of water solution with 2% w/V DMSO have been added to the first aliquot, and 100 µl of the solution containing CB have been added to the second and third ones. The three blood aliquots have been placed once more in the thermostat for 60 minutes at 37 °C.

Subsequently, for each blood aliquot treated in this way, 30 µl have been taken and added to tubes containing 4 ml of saline solution with known concentration corresponding to specific concentrations of NaCl (0.9%, 0.7%, 0.6%, 0.55%, 0.5%, 0.45%, 0.4%, 0.3% and bidistilled water). Such solutions have been prepared according to the medical protocol by Haut *et al.* (1962). Test tubes were gently mixed by inversion and allowed to stand in the thermostat for 30 minutes at the temperature of 37 °C to undergo osmotic lysis. The suspensions were centrifuged at 3 000 RPM for 10 minutes at 4 °C to separate non-hemolyzed cells and ghosts. The supernate was decanted and its optical density was determined by UV-visible spectroscopy at 540 nm. According to the maximum hemolysis obtained in bidistilled water, each absorbance value in every single measurement has led to the calculation of the percentages of relative hemolysis which were plotted versus the respective salt concentrations (Figure 2). Essentially, the method by Haut *et al.* (1962), later modified by O'Dell *et al.* (1987), has been used in order to evaluate the osmotic fragility.

As concerns the work methodology which has led to the preparation of plasma on which laboratory tests have been carried out, blood samples have been taken according to the same procedures above mentioned. In this case, blood was collected into sterile test tubes containing EDTA and temporarily maintained at 4 °C.

A first group of 10 blood samples has been divided into two aliquots of 1 ml: 100 µl of water solution with 2% w/V DMSO have been added to the first aliquot, and 100 µl of the solution containing CB have been added to the second one. The two blood aliquots have then

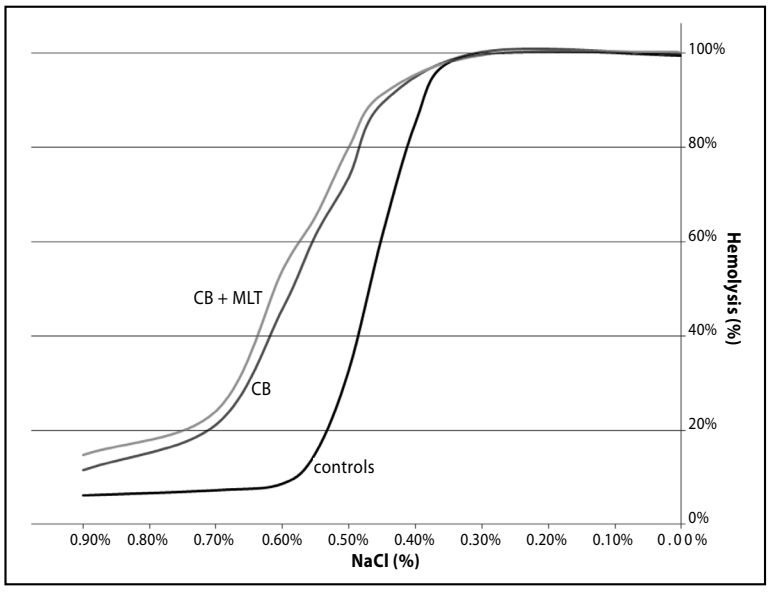

Fig. 2. Osmotic fragility curves.

been put in the thermostat for 60 minutes at 37 °C. The samples were centrifuged at 3000 RPM for 10 minutes at 4°C. Plasma obtained in this way has been separated and stored at –20 °C.

A second group of 10 blood samples has been divided into two aliquots of 1 ml: 40 µl of water solution with 2% w/V DMSO have been added to the first aliquot, and 40 µl of the solution containing MLT have been added to the second one. The two blood aliquots have then been put in the thermostat for 30 minutes at 37°C. Subsequently, 100 µl of the solution containing CB have been added to both aliquots, and again placed in the thermostat for 60 minutes at 37 °C. Afterwards, the samples were centrifuged at 3 000 RPM for 10 minutes at 4 °C. Plasma obtained has been separated and stored at –20 °C.

The samples obtained in this way have been analysed for the research of the following tests: LDH, tested according to IFCC method, plasma total phospholipids, tested according to spectrophotometric method and MDA, tested according to HPLC method.

Statistical analysis of data was carried out using a two-tailed Student t-test for unpaired data. Any variations with $p<0.05$ were considered significant.

RESULTS

Figure 2 shows three osmotic fragility curves. Compared to the physiological conditions (black line), the CB treatment (dark grey line) considerably decreases the globular resistance. The CB + MLT treatment (light grey line) does not modify the general trend of the osmotic fragility curve concerning the CB, showing, at the same time, a clear increase in the hemolytic phenomenon in all considered points.

Table 1 shows the average percentages of hemolysis of 10 samples concerning three different treatments. With reference to the control samples, the table indicates the variations in percentages concerning the CB and CB + MLT treatments in 4 out of 9 reference points.

Table 2 shows the LDH values, plasma total phospholipids and MDA pertaining to the 10 control samples compared to those treated with CB.

Table 3 indicates the LDH, plasma total phospholipids and MDA values referred to 10 samples only treated with CB compared to those treated with CB + MLT.

It can be noted that CB considerably increases the LDH value (Figure 3) compared to the control samples: +85% ($p<0.0001$). The plasma total phospholipids level remains very similar and no significant variation occurs. The MDA level after the CB treatment rises by 7.9% which is not statistically significant.

If compared to the samples treated with CB, the LDH values of the samples treated with CB + MLT seem to be increased by 18.7% (Figure 4). Such a variation concerning the first 8 samples, shows a rise of 41.4% with a high significance level ($p<0.02$). The analyses of the samples treated with CB + MLT compared to the control samples show a 105% increase of LDH values with $p<0.0001$.

Also in this second series of data, the plasma total phospholipids values do not vary significantly between the two groups. Similarly, the MDA levels between the group treated with CB + MLT and the one treated only with CB do not show substantial variations, which is a phenomenon related to the reducing reaction conditions.

DISCUSSION

Some interesting remarks based on the data above mentioned may be pointed out: as it was predictable, CB substantially decreased the osmotic globular resistance. Such an action could be due to a remarkable increase of deformation phenomena owing to the loss of the actinic component of the erythrocyte membrane-CSK (Lin & Lin 1978; Beck *et al.* 1972; Tsukita *et al.* 1984) whose loss causes a significant rise of the plastic phenomena of red blood cells. This is clearly noticeable by analysing

Tab. 1. Average percentages of hemolysis of the three different treatments and percentage variations compared to the control samples referred to the treatments with CB and CB + MLT.

NaCl concentration	Controls	CB	CB + MLT
0.90%	6.10%	11.53% (+89%; $p<0.01$)	14.66% (+140%; $p<0.01$)
0.70%	7.22%	21.19% (+193%; $p<0.01$)	24.01% (+233%; $p<0.01$)
0.60%	8.60%	45.76% (+432%; $p<0.0001$)	53.95% (+527%; $p<0.0001$)
0.50%	32.81%	73.70% (+125%; $p<0.001$)	80.14% (+144%; $p<0.0001$)

Tab. 2. Plasma analysis of 10 control samples compared to those treated with CB.

Test	Controls	CB
LDH (U/l)	774.6 ± 68.1	1431.2 ± 81.6
Plasma Phospholipids (mg/100 ml)	122.5 ± 4.9	126.4 ± 6.3
MDA (µg/l)	17.7 ± 0.9	19.1 ± 1.1

Tab. 3. Plasma analysis of 10 samples only treated with CB compared to those treated with CB + MLT.

Test	CB	CB + MLT
LDH (U/l)	1337.2 ± 155.9	1586.8 ± 98.3
Plasma Phospholipids (mg/100 ml)	121.1 ± 4.5	128.9 ± 5.2
MDA (µg/l)	13.8 ± 1.1	12.7 ± 1.5

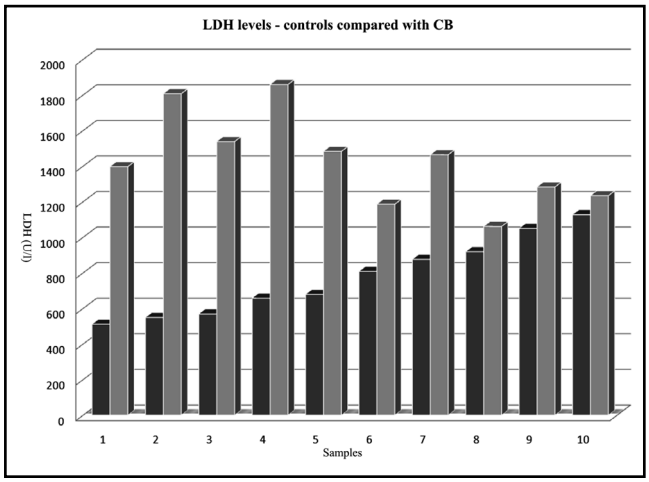

Fig. 3. LDH of 10 control samples compared to those treated with CB.

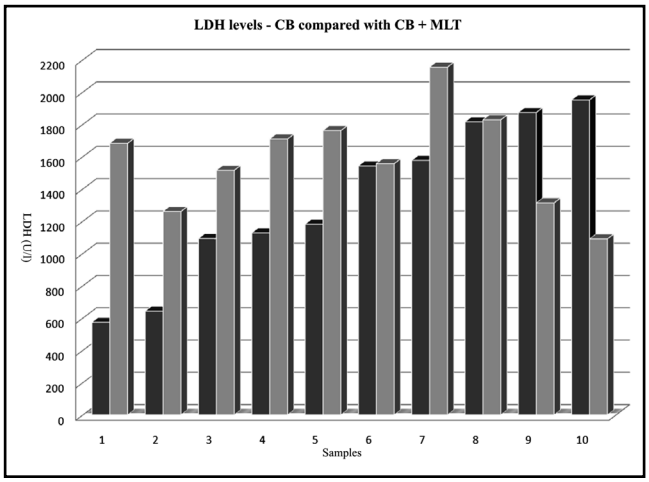

Fig. 4. LDH of 10 samples only treated with CB compared to those treated with CB + MLT.

the globular osmotic resistance curve which, compared with the control samples, appears to move considerably to the left. The curve of the samples treated with CB shows a sharp increase of the hemolytic phenomena in all points taken into consideration, which is also obvious in the difference in the first flex point, if compared with the curve of the control samples. Besides that, it is also evident as the overall curve acquires the trend of a typical microcytic hemolytic anaemia, showing the distinctive trend of hemolytic pathologies caused by structure defects namely the performance and splicing of the cytoskeletal proteins.

MLT treatment does not hinder the action of CB, on the contrary, it increases both its power and effectiveness. The globular osmotic resistance curve of the samples treated with CB + MLT seems to be moved to the left compared with the samples only treated with CB: the percentages of hemolysis in the different points are constantly and evenly increased. This last curve shows the typical trend of hemolytic anaemia, namely in the physiopathological processes in the cytoskeletal component of the erythrocyte membrane.

Plasma levels of LDH considered as a hemolytic marker (Kato *et al.* 2006) indicate, in general, a considerable and statistically significant increase of the hemolysis percentage in the CB treated samples compared with the controls. Also the values of LDH of the group of rats treated with CB + MLT compared to those treated only with CB are statistically and significantly higher in 8 samples out of 10. Finally, the samples regarding the CB + MLT action show an increase of LDH values compared with the control samples of 105% with $p<0.0001$. If the percentage of hemolysis in the samples treated with CB + MLT has actually risen from the analysis of LDH values, we may deduce that such an effect, at least under the adopted conditions, does not require Ca^{2+} ions which have been trapped by the chelating agent.

CB treatment compared with the control samples slightly increases the MDA levels and it is not statistically significant. These levels are homogeneous in all treated samples, especially because of the reducing conditions adopted. This suggests that for the appearance of the CB effects the oxidoreductive processes of the erythrocyte membrane lipids are not involved. Similarly, the observed phenomena of the MLT treated samples do not seem to be caused by its reducing power. The levels of plasma phospholipids appear to be almost homogeneous for all treated groups which suggests that there have not been significant variations of the membrane phospholipid component.

From the above mentioned data, we can draw the conclusion that MLT increases in power and effect the CB action on the microfilaments of the erythrocyte membrane. On the one hand, this phenomenon appears to be obvious and probable, but on the other hand, it is difficult to identify the mechanism through which it expresses itself, as well as it is hard to establish its timelines. However, it is possible to suggest three possible mechanisms through which MLT can increase the destabilising action on the microfilaments performed by CB. This action could be carried out by G-protein coupled receptors and, in particular, through MEL-1 or MEL-2 (Benítez-King 2006), which could be the result of the MLT action on one of the kinases associated with the Rho-kinase family, or it could also be an action on the calmodulin (Benítez-King & Antón-Tay 1993; Benítez-King *et al.* 1993; Ramírez-Rodríguez 2003; Ramírez-Rodríguez *et al.* 2007; Ortíz-López *et al.* 2009; Reiter *et al.* 2010). It is also likely that MLT could affect the microfilaments and/or the G-actin monomers, bonding to them (Finocchiaro & Glikin 1998). This is understandable if we consider that MLT has a small molecular structure that, at the same time, can act as both receptor and donator of hydrogen bonds, as well as operating through π aromatic interactions. It is also

interesting to notice that MLT seems to influence the hexokinase action and also other enzymes involved in the glycolytic pathway (Martins *et al.* 1998; Frangioni *et al.* 2003; Akmali *et al.* 2010) (Figure 5). To this purpose, it should be reminded that the G-actin has a domain homologous to hexokinase, and with variable degree it also has a domain homologous to other enzymes involved in the Embden-Meyerhof-Parnas pathway (Kabsch *et al.* 1990; Kabsch & Holmes 1995; Wilson & Schwab 1996).

It is worth noting that the G-actin and the genes which codify its isoforms are extremely well-preserved throughout the evolution scale, presenting slight differences even between species seemingly diverse and distant. All this suggests that the actin has an important evolutive and phylogenetic connection. Similarly, MLT is a substance which is ubiquitously spread and as well as the genes which direct the expression of the enzymes responsible for its synthesis: they are present in both plant and animal life, both as metazoans and non-metazoans, suggesting important implications also for MLT in the phylogenesis and in the molecular evolution. Furthermore, considering its presence also in non-metazoans, it has a biological role which goes beyond the simple regulation of circadian rhythms. Therefore, it is very likely that MLT which appeared on the molecular scenario in extremely ancient times, far before the appearance of other molecular systems such as the receptor and enzyme ones, could interact with the actin and actin microfilaments, with which it shares important evolution bonds and molecular phylogenesis (Hardeland, 1999). It cannot be excluded that there might be more than a mechanism relating to the observed phenomena and that these may not be mutually incompatible.

It is very interesting to note that many actions of MLT described in the literature may find their molecular reason in a MLT action on the CSK: this seems particularly obvious if we consider that the CSK is very important in most cell processes, amongst which the cell growth and differentiation. An action on the CSK may also be involved in part of its oncostatic and prodifferentiative mechanisms.

As concerns its activity on blood and, more precisely, on the corpuscular component, we may propose important observations: the rheological properties of blood are directly correlated to the number of erythrocytes and their geometric shape. For each mm^3 of blood, 3.5–5.5 × 10^6 erythrocytes, 4.5–10 × 10^3 leukocytes and approximately 1.5–4 × 10^5 platelets are present on average. To sum up, there is a leukocyte and 50–100 platelets for 1 000 erythrocytes. Platelets however, considering their small volume, are not significant from the rheological point of view. Similarly, the leukocytes are not significant owing to their modest number. More precisely, the hematocrit, which is the volumetric concentration of the blood corpuscular fraction, could be likened to the volumetrical fraction of red blood cells. From a rheological viewpoint, while the plasma can be considered a fluid with a Newtonian behaviour, the whole blood cannot be likened to a Newtonian fluid as it presents a pseudoplastic behaviour. Ultimately,

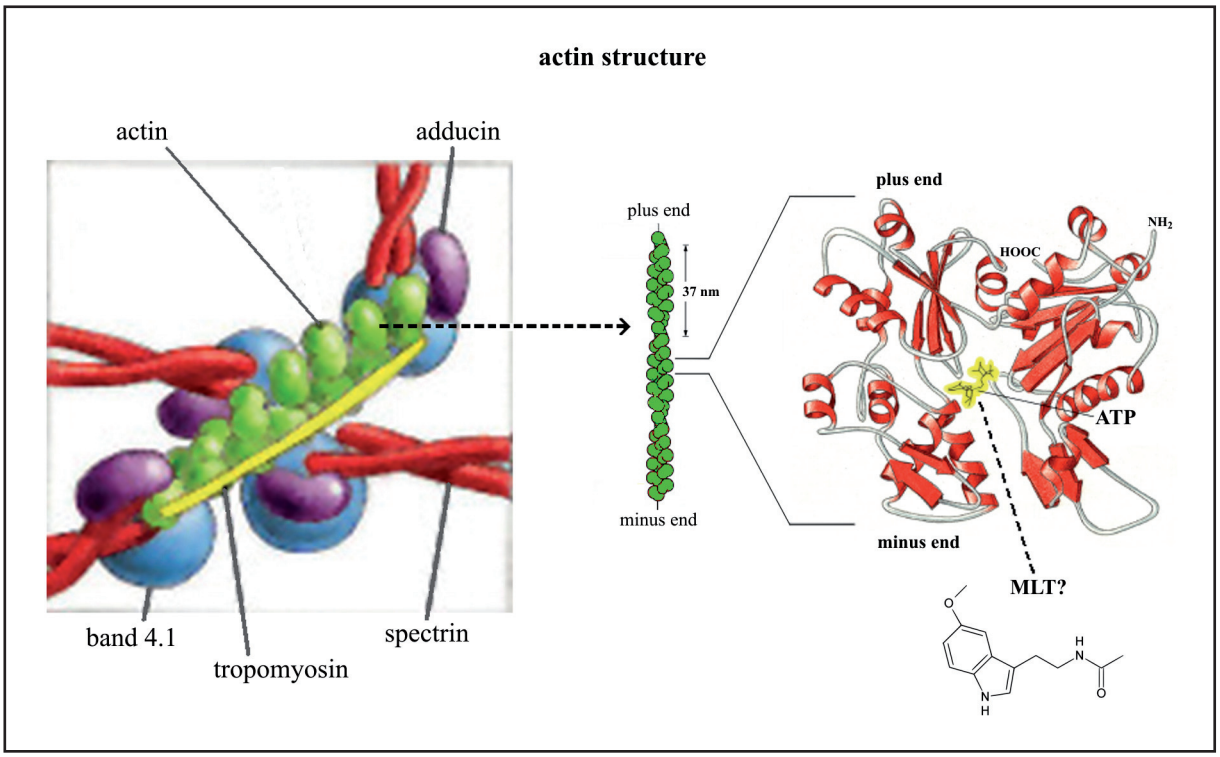

Fig. 5. Essential elements of the actin structure.

the total viscosity of the whole blood (ηblood) is the addition of the erythrocyte viscosity (ηRBC) and the plasma viscosity (ηplasma). Therefore, considering that the plasma viscosity can be compared to a constant included between 1.2–1.9 cP, it can be understood that the blood viscosity is a function of the erythrocyte viscosity in relation with three main variables: the volumetric fraction of red blood cells or hematocrit, the rouleaux formation and the erythrocyte deformability.

The observations above mentioned however pertain the blood circulation in vessels of small diameter and, in particular, in metarterioles and capillaries with diameter of only 3 μm and in small veins and vessels of small-medium size, whereas in larger vessels, such as for example the aorta, the blood behaviour in reality is not pseudoplastic, but Newtonian. Therefore, it is understandable how important the relation between the blood viscosity and the efficiency of microcirculation is. The blood viscosity increases considerably with the decrease of deformability: this characteristic of red blood cells is extremely important and rests on the characteristics of the membrane-CSK system of the erythrocytes, on the viscosity of the internal liquid and the ratio volume/superficial area (Schmid-Schonbein *et al.* 1969; Chien 1977).

An intervention of MLT on the erythrocyte CSK and, in particular, on the actinic microfilaments could be sensibly postulated especially taking into account the following considerations. If we consider that CB, which due to its powerful destabilising action on the microfilaments, greatly increases the erythrocyte deformability. Therefore, as a consequence, it considerably reduces the osmotic fragility. Furthermore, if we consider that MLT in low oxidative stress conditions is able to increment the power and effectiveness of the CB effect, and finally, if we analyse the overall observations above-mentioned.

Therefore, since MLT is synthesised and/or gathered in the platelets, and *in vitro* the erythrocytes are able to convert serotonin into MLT. Many other cells in the immune-hematological and in the immune-hematopoietic systems are able to synthesise MLT as an autocrine/paracrine factor also independently from the photoperiod. Therefore, it cannot be underestimated its important role in the physiology of the microcirculation where it may actively contribute to the complex regulation of the blood viscosity through the modulation of the phenomena of erythrocyte plasticity.

CONCLUSION

From the data and observations above mentioned we can resolve that, under followed conditions, MLT increases the power and effectiveness of CB action on the red blood cells, supposedly thanks to an action on the actinic microfilaments and on the CSK. These may represent a new action target for MLT. Additional and further studies will be necessary in order to fully understand the phenomena previously explained. It appears however possible that MLT, due to its actions, may play an active role in the control of the rheological characteristics and in the system of blood circulation as well as in the modulation of the erythrocyte plastic phenomena.

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