Prooxidant activity of melatonin promotes fas-induced cell death in human leukemic Jurkat cells

Albert Wölfler[a,b], Hans-Christian Caluba[c], Peter M. Abuja[d], Gottfried Dohr[c], Konrad Schauenstein[b], Peter M. Liebmann[b,*]

[a] *Department of Medicine, Division of Hematology, University of Graz, Auenbruggerplatz 38, A-8036 Graz, Austria*
[b] *Institute of Pathophysiology, University of Graz, Heinrichstrasse 31a, A-8010 Graz, Austria*
[c] *Institute of Histology and Embryology, University of Graz, Harrachgasse 21, A-8010 Graz, Austria*
[d] *Institute of Biochemistry, University of Graz, Halbärthgasse 5/1, A-8010 Graz, Austria*

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Abstract The antioxidant activity of melatonin (MEL) has been considered to constitute part of its physiological as well as pharmacological effects. However, as described herein we found a profound prooxidant activity of micro- to millimolar concentrations of MEL in the human leukemic Jurkat cell line. This prooxidant effect was increased in glutathione-depleted cells and counteracted by antioxidants. As a consequence MEL promoted fas-induced cell death. These data therefore indicate that MEL may be a modulator of the cellular redox status, but does not necessarily act as an intracellular antioxidant.

Key words: Melatonin; Reactive oxygen species; Lymphocyte apoptosis; Cellular redox regulation

1. Introduction

The pineal hormone melatonin (MEL) has been shown to exhibit distinct antioxidant features in vitro as well as in vivo (reviewed in [1]). Based on these findings, MEL was considered to exert its physiological and pharmacological effects at least partly via its antioxidant activity [2]. Furthermore, MEL was suggested to have therapeutic implications in deferring aging processes [1,2], where reactive oxygen species (ROS) are known to play an important role [3]. More recently, however, only a limited antioxidant activity of MEL was observed in several systems [4–7].

Besides promoting deleterious effects in high concentrations, ROS function as intracellular downstream messengers targeting specific proteins and genes [8–10]. For example, programmed cell death in lymphocytes is known to be influenced by alterations of the cellular redox state as well as by intracellular ROS formation [11–16]. Since little is known about effects of MEL on the intracellular redox state, even though MEL can easily cross cell membranes due to its amphiphilicity [17], we examined whether MEL interferes with intracellular ROS production in fas-induced cell death in Jurkat cells, a human leukemic T-cell line, which constitutively expresses the fas receptor [18].

2. Materials and methods

2.1. Drugs and cell cultures

If not otherwise specified, reagents were purchased from Sigma (St. Louis, MO, USA) and were of analytical grade or better. The human leukemic T-cell line Jurkat E6.1 was obtained from the European Collection of Animal Cell Cultures and cultured in RPMI1640 medium with 10% fetal calf serum (FCS) (both from PAA Laboratories, Exton, USA), 100 IU/ml penicillin (Biochemie, Vienna, Austria) and 100 μg/ml streptomycin. For experimental studies, cells were washed twice in PBS (10 mM sodium phosphate, 160 mM NaCl, pH = 7.4) and resuspended in RPMI1640 at a concentration of 10^6 cells/ml, unless otherwise mentioned. Cell viability as determined with trypan blue exclusion test and propidium iodide staining and FACS analysis was $>98\%$.

MEL was dissolved in ethanol to give a 500 mM stock solution, which was further diluted in RPMI1640 to give a 1 mM (for one experimental setup 2.5 mM) concentration resulting in a 0.2% (0.5% respectively) ethanol concentration after added to the cells. Further dilutions of MEL were done with medium containing 0.2% (0.5%) ethanol, and cells of the control group were suspended in medium containing 0.2% (0.5%) ethanol. Therefore, all experimental groups were exposed to the same amount of ethanol.

2.2. Determination of ROS formation in resting Jurkat cells

Formation of ROS was monitored using the oxidation sensitive dye dihydrorhodamine 123 (DHR, Molecular Probes, Eugene, OR, USA), which is the uncharged and non-fluorescent reduction product of the cationic fluorescent dye rhodamine 123. Jurkat cells (2×10^6 cells/ml) were incubated in RPMI in the presence of 2 μM DHR for 10 min at 37°C. After washing, cells were resuspended in RPMI (10^6/ml) and left untreated or incubated with various concentrations of MEL. At the indicated intervals, rhodamine fluorescence was analyzed at 488 nm/525 nm (excitation/emission wavelength) using a FACScan cytofluorometer (BD).

2.3. Determination of hydrogen peroxide

Since antioxidants may interact with cell culture media to generate hydrogen peroxide [19,20], we determined hydrogen peroxide formation in RPMI1640 medium after addition of up to 2 mM MEL by oxygen measurement in the presence of 1000 U/ml catalase using a CLARK electrode in a thermostatted, stirred setup (Oxygraph, Anton Paar, Graz, Austria). As a positive control, hydrogen peroxide (250 μM) was used.

2.4. Modulation and determination of intracellular glutathione (GSH)

After 24 h incubation of Jurkat cells (1×10^6 cells/ml) with various

*Corresponding author. Fax: (43)-316-380 9640; internet: http://www.kfunigraz.ac.at/fupwww/liebmann.html.
E-mail address: peter.liebmann@kfunigraz.ac.at (P.M. Liebmann).

Abbreviations: ROS, reactive oxygen species; MEL, melatonin; DHR, dihydrorhodamine; mAb, monoclonal antibody; FCS, fetal calf serum; GSH, glutathione; BSO, buthionine-sulfoximine; EGSH, glutathione-ethylester; HE, hydroethidium; TX, trolox

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(1; 2.5; 10; 25; 100; 250 μM) concentrations of buthionine-sulfoximine (BSO), an inhibitor of intracellular GSH synthesis [13], cells were washed twice in PBS and either stained with DHR according to the above-mentioned protocol or used for determination of cellular GSH concentration by the method described by Tietze [21] using a commercially available microtiter plate assay (Dojindo Laboratories, Japan). This assay was performed according to the manufacturer's instructions.

2.5. Determination of anti-fas-induced intracellular ROS formation and cell death

Fas-induced programmed cell death was induced using the anti-fas monoclonal antibody (mAb) CH-11 (Immunotech, Vienna, Austria) [22]. Since active mitochondria are necessary for determination of ROS formation with DHR, but programmed cell death is associated with dysregulation of mitochondrial functions [23], hydroethidium (HE), which is the oxidation sensitive reduction product of the DNA binding dye ethidium, was used to assess ROS formation in Jurkat cells undergoing fas-induced apoptosis. A protocol was used according to Zamzami et al. [23] with slight modifications: Briefly, Jurkat cells (2×10^6 cells/ml) were incubated in RPMI in the presence of 2 μM HE for 10 min at 37°C. After washing, cells were resuspended in RPMI (2.5×10^5/ml) and incubated with 100 ng/ml anti-fas mAb CH-11 and various concentrations of MEL or glutathione-ethylester (EGSH), which is a membrane-permeable form of GSH. After 6 h, ethidium fluorescence was analyzed at 488 nm/600 nm (excitation/emission wavelength) with the FACScan cytofluorometer.

The percentage of cells undergoing apoptosis was determined using annexin V-FITC (Pharmingen, San Diego, USA) binding and propidium iodide (PI) staining, as previously described by Vermes et al. [24]. Jurkat cells (2.5×10^5 cells/ml) were simultaneously incubated with the anti-fas mAb (100 ng/ml) and various concentrations of MEL, EGSH and trolox (TX), a water-soluble analogue of α-tocopherol. After 4 h cells were washed twice with ice-cold HEPES buffer (10 mM HEPES/NaOH; 140 mM NaCl; 2.5 mM $CaCl_2$; pH 7.4), and the pellet was incubated with 2 μl annexin V-FITC (1 mg/ml) and 10 μl PI (50 μg/ml) for 15 min at room temperature according to the manufacturer's instructions. The percentage of annexin V-FITC positive and PI negative cells was analyzed in the FACScan at an emission wavelength of 525 nm and 600 nm, respectively.

In a second approach, Jurkat cells were preincubated either with MEL, TX, EGSH alone, or MEL plus TX, or MEL plus EGSH for 4 h. After thorough washing to remove the drugs, cells were treated with anti-fas mAb (100 ng/ml) for an additional 4 h. Analysis of the percentage of apoptotic cells was done as described above.

2.6. Statistical analysis

Using the SigmaStat 2.03 software package a repeated measures ANOVA followed by a Tukey post-hoc analysis was calculated.

3. Results

3.1. Melatonin exerts prooxidant activity in resting Jurkat cells

In contrast to TX, which decreased intracellular rhodamine fluorescence in resting Jurkat cells loaded with DHR (see Fig. 1B), micro- to millimolar concentrations of MEL enhanced it in a dose- and time-dependent manner (see Fig. 1). In order to exclude hydrogen peroxide formation in RPMI cell culture medium, which has been shown for some antioxidants [19,20], we measured hydrogen peroxide formation after MEL addition as described in Section 2 in a cell-free system: no hydrogen peroxide formation was detected (data not shown). To exclude a possible selenium deficiency in long-time cultured Jurkat cells to be responsible for the observed prooxidant activity of MEL, Jurkat cells were preincubated for 48 h in full medium containing 1 nm to 1 μM sodium selenite, which did not change the results (data not shown). Since indoleamines in general are known to influence the cellular redox state [6,25], we investigated whether this prooxidant effect is specific for MEL. In contrast to MEL, *N*-acetylserotonin (NAS), which is the immediate precursor of MEL, slightly but significantly decreased intracellular ROS formation (data not shown), indicating that this prooxidant effect might be specific for MEL.

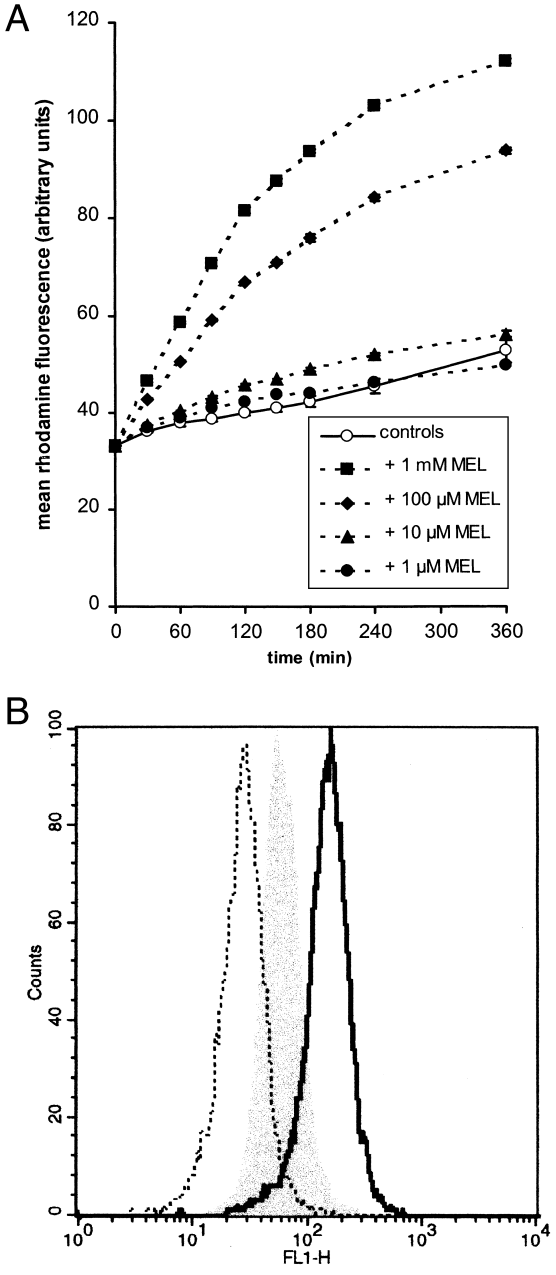

Fig. 1. A: Increased intracellular ROS formation in Jurkat cells after MEL treatment. Jurkat cells (2×10^6 cells/ml) were loaded with 2 μM DHR for 10 min at 37°C. After washing with PBS, cells were resuspended in RPMI (1×10^6/ml) and incubated with the indicated concentrations of MEL. After the indicated time periods, rhodamine fluorescence was analyzed using a Becton-Dickinson FACScan. 10 μM, 100 μM and 1 mM MEL led to a dose- and time-dependent increase in intracellular ROS formation ($P<0.001$ vs. controls at each time point except 10 μM MEL after 30 min (not significant) and 60 min ($P<0.05$)). 1 μM MEL differed significantly vs. controls after 90, 120 and 150 min ($P<0.05$). Data represent mean ± S.E.M. of triplicates ($n=5$). B: Rhodamine fluorescence (FL1) histogram of MEL-treated and TX-treated Jurkat cells. While after 120 min of incubation TX decreased intracellular rhodamine fluorescence (dotted line) compared to controls (gray area), MEL increased it (thick line). FL1 denotes rhodamine fluorescence.

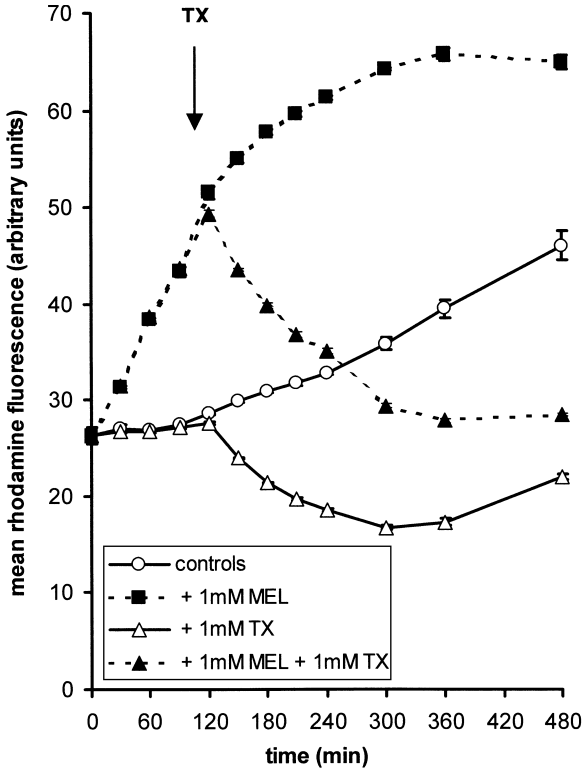

Fig. 2. Reversal of MEL-induced intracellular ROS formation by addition of TX. After loading with DHR, Jurkat cells were incubated with/without 1 mM MEL. After 2 h 1 mM TX was added. Addition of TX counteracted MEL-induced intracellular ROS formation ($P < 0.001$). Data represent mean ± S.E.M. of triplicates of one experiment out of three giving identical results.

As shown in Fig. 2, TX was able to completely neutralize MEL-induced ROS in Jurkat cells. Furthermore, preincubation of Jurkat cells with various concentrations of BSO, which inhibits intracellular GSH synthesis [13], showed that the prooxidant effect of MEL is dependent on intracellular GSH: the lower the cellular GSH concentration, the more pronounced the prooxidant activity of MEL (Fig. 3). These results corroborate that the observed increase in intracellular rhodamine fluorescence is indeed due to a prooxidant cellular activity of MEL.

3.2. MEL promotes fas-induced cell death

In order to assess the functional relevance of these findings, we determined the effect of MEL on fas-induced cell death in Jurkat cells: As outlined in Table 1, EGSH was able to decrease the percentage of cells undergoing apoptosis in a dose-dependent manner, which is in good agreement with results of Williams et al. [12]. This was accompanied by an attenuation of fas-induced intracellular ROS formation, as assessed using the oxidation sensitive dye HE (see Table 1). In contrast, MEL dose dependently enhanced the percentage of cells undergoing fas-induced apoptosis and increased fas-induced intracellular ROS formation. Without anti-fas mAb incubation neither MEL nor EGSH influenced the percentage of annexin V binding cells.

Preincubation of Jurkat cells with MEL increased their susceptibility to fas-induced apoptosis (Fig. 4). Concomitant addition of TX as well as of EGSH abolished the effect of MEL. Interestingly, in contrast to EGSH, preincubation with TX alone had no influence on the percentage of cells undergoing fas-induced cell death, again suggesting that only thiol-containing antioxidants, like EGSH, can modulate fas-induced apoptosis [12]. Furthermore, this finding suggests the involvement of different ROS in fas activation and MEL-induced intracellular oxidation processes.

4. Discussion

Our results clearly demonstrate a prooxidant cellular activity of MEL in Jurkat cells. MEL-induced ROS formation was more pronounced in GSH-depleted cells and was counteracted by TX. Among indoleamines, this prooxidant effect might be specific for MEL, since *N*-acetylserotonin, which is the immediate precursor of MEL and has also been described to exert antioxidant properties [6], decreased intracellular ROS formation in our experimental setup.

These results conflict with reports of antioxidant properties of MEL in lymphocytes and macrophages [6,26–28]. However, Barsacchi et al. [29] showed that micromolar concentrations of MEL exerted prooxidant effects in human erythrocytes exposed to oxidative stress. These results were confirmed recently by Medina-Navarro et al. [30], who reported prooxidant properties of MEL in the in vitro interaction with singlet oxygen. Differences in the redox state and the susceptibility to oxidant-induced changes in cell functions among different cell types [31–33] most likely explain the conflicting results. This

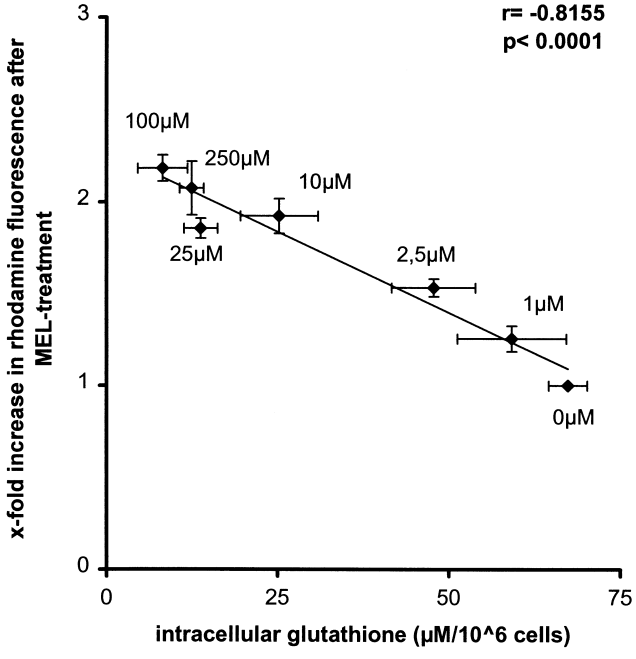

Fig. 3. Correlation between intracellular GSH content and prooxidant activity of MEL in Jurkat cells. After incubation with various BSO concentrations (1–250 µM) for 24 h, Jurkat cells were either loaded with DHR, exposed to MEL (1 mM) for 2 h and analyzed for their rhodamine fluorescence, or their intracellular GSH concentration was determined as described in Section 2. A highly significant negative correlation between intracellular GSH content (expressed in µM per 10^6 cells) and extent of prooxidant activity of MEL was found. Data represent results from two independent experiments, each done in duplicate (mean ± S.E.M. for the different BSO concentrations indicated).

Table 1
Influence of MEL and EGSH on fas-induced cell death and its correlation to enhanced intracellular ROS formation in Jurkat cells

Treatment	Percent cells undergoing apoptosis		Intracellular ROS concentration	
Controls	5.35 ± 0.50		42.5 ± 0.71	
+Anti-fas mAb	22.11 ± 0.80		59.09 ± 0.29	
	MEL	EGSH	MEL	EGSH
0.01 mM	22.34 ± 0.34	ND	ND	ND
0.1 mM	24.62 ± 0.18	ND	63.51 ± 0.78***	ND
0.5 mM	30.80 ± 0.48***	21.73 ± 0.03	ND	ND
1 mM	37.28 ± 0.24***	19.73 ± 0.40*	65.97 ± 0.48***	56.28 ± 0.33*
2.5 mM	43.60 ± 1.63***	17.85 ± 0.18**	68.51 ± 0.77***	55.56 ± 0.48**
5 mM	ND	12.34 ± 0.04***	ND	50.26 ± 0.23***

*$P < 0.05$ vs. anti-fas mAb; **$P < 0.01$ vs. anti-fas mAb; ***$P < 0.001$ vs. anti-fas mAb; ND denotes not determined.
Jurkat cells (2.5×10^5/ml) were incubated with the indicated concentrations of MEL and EGSH in the presence of 100 ng/ml anti-fas mAb. After 4 h, cells were washed twice with ice-cold HEPES buffer and the pellet was incubated with 2 μl annexin V-FITC (1 mg/ml) and 10 μl PI (50 μg/ml) for 15 min at room temperature. The percentages of annexin V positive/PI negative cells were analyzed using a Becton-Dickinson FACScan. Concomitantly, Jurkat cells (2×10^6 cells/ml) were loaded with 2 μM HE for 10 min at 37°C. After washing with PBS, cells were resuspended in RPMI/10% FCS (2.5×10^5/ml), and incubated with the indicated concentrations of MEL and EGSH in the presence of 100 ng/ml anti-fas mAb. After 6 h, ethidium fluorescence was analyzed using a Becton-Dickinson FACScan. Data represent mean ± S.E.M. of triplicates ($n = 3$).

notion is supported by the fact that ascorbic acid also increased DHR oxidation in our Jurkat cell model (data not shown). Ascorbic acid is known to exhibit antioxidant as well as prooxidant properties both in vitro [33–36] and in vivo [37,38].

Functional consequences of the prooxidant effect of MEL in Jurkat cells became obvious by the promotion of fas-induced cell death. In human-activated peripheral blood T-cells as well as in Jurkat cells, fas-induced apoptosis was shown to be preceded by an increase in intracellular ROS formation [15], and thiol-based antioxidants, such as GSH and *N*-acetylcysteine, as well as inhibition of GSH synthesis by BSO, influenced fas-mediated cell death [12,13]. Pre- or coincubation with MEL, which both lead to an intracellular prooxidant state, thus may render the cells more susceptible to stimuli of cell death, as has recently been shown to be responsible for increased human T-cell leukemia virus-I (HTLV-I) tax-mediated apoptosis in Jurkat cells [39]. Increased intracellular ROS formation may also modulate the expression of the fas receptor, since antioxidants can down-regulate fas receptor expression on the surface of lymphocytes [40], and oxidants, such as hydrogen peroxide, have been shown to increase fas receptor expression in cultured human endothelial cells [41].

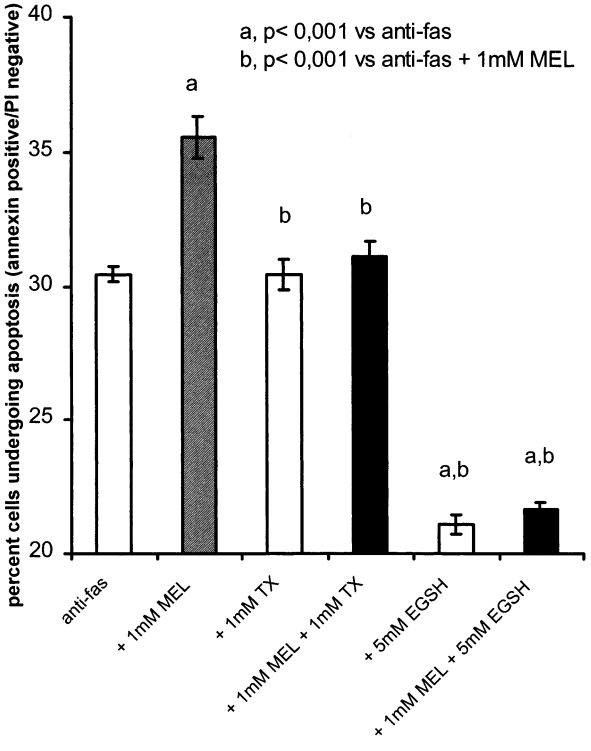

Fig. 4. Reversal of MEL-induced enhancement of fas-mediated cell death in Jurkat cells by TX and EGSH. Jurkat cells (2.5×10^5/ml) were incubated with MEL alone, TX alone, EGSH alone, or MEL and TX, or MEL and EGSH. 4 h later, cells were washed thoroughly to remove the drugs, resuspended in RPMI/10% FCS, and incubated with 100 ng/ml anti-fas mAb. After a total of 8 h, the percentage of apoptotic cells was determined. Data represent mean ± S.E.M., $n = 3$, each done in triplicate.

Concerning the influence of MEL on programmed cell death, most data are available from experiments using neuronal cells: While some groups reported protection against programmed cell death in neuronal cells and cell lines by antioxidant activity of MEL [42–45], Harms et al. recently demonstrated that micro- to millimolar concentrations of MEL are protective against necrotic cell death but can exaggerate cell damage in resting primary neuronal cultures as well as after induction of apoptosis [46]. In malignant cells, such as the human breast cancer cell line MCF-7, micromolar concentrations of MEL have recently been reported to induce apoptosis [47]. Furthermore, MEL was reported to enhance programmed cell death in a murine colon cancer model in vivo [48]. The respective mechanisms for these apoptosis-promoting effects are unknown so far.

To summarize our presented data show that MEL can exhibit strong prooxidant activity in Jurkat cells, which leads to the promotion of fas-induced cell death. Therefore, MEL may be a modulator of the cellular redox status in both ways, and is not necessarily protecting against oxidative hazard. The premises determining pro- or antioxidant effects of melatonin in cellular systems as well as the underlying mechanisms of action have to be clarified in further studies.

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References

[1] Reiter, J.R. (1998) Prog. Neurobiol. 56, 359–384.
[2] Reiter, R.J., Oh, C.S. and Fujimori, O. (1996) Trends Endocr. Metab. 7, 22–27.
[3] Halliwell, B. (1994) Lancet 344, 721–724.
[4] Marshall, K.A., Reiter, J.R., Poeggeler, B., Arouma, O.I. and Halliwell, B. (1996) Free Radic. Biol. Med. 21, 307–315.
[5] Abuja, P.M., Liebmann, P.M., Hayn, M., Schauenstein, K. and Esterbauer, H. (1997) FEBS Lett. 413, 289–293.
[6] Wölfler, A., Abuja, P.M., Schauenstein, K. and Liebmann, P.M. (1999) FEBS Lett. 449, 206–210.
[7] Montaldo, C., Cannas, E., Dettori, T., Congiu, L. and Atzori, L. (2000) Life Sci. 66, PL339–344.
[8] Finkel, T. (1998) Curr. Opin. Cell. Biol. 10, 248–253.
[9] Allen, R.G. and Tresini, M. (2000) Free Radic. Biol. Med. 28, 463–499.
[10] Finkel, T. (2000) FEBS Lett. 476, 52–54.
[11] Sato, N., Iwata, S., Nakamura, K., Hori, T., Mori, K. and Yodoi, J. (1995) J. Immunol. 154, 3194–3203.
[12] Williams, M.S. and Henkart, P.A. (1996) J. Immunol. 157, 2395–2402.
[13] Chiba, T., Takahashi, S., Sato, N., Ishii, S. and Kikuchi, K. (1996) Eur. J. Immunol. 26, 1164–1169.
[14] Bauer, M.K.A., Vogt, M., Los, M., Siegel, J., Wesselborg, S. and Schulze-Osthoff, K. (1998) J. Biol. Chem. 273, 8048–8055.
[15] Banki, K., Hutter, E., Gonchoroff, N.J. and Perl, A. (1999) J. Immunol. 162, 1466–1479.
[16] Clement, M.V. and Pervaiz, J. (1999) Free Radic. Res. 30, 241–252.
[17] Menendez-Pelaez, A., Poeggeler, B., Reiter, R.J., Barlow-Walden, L.R., Pablos, M.I. and Tan, D.X. (1994) J. Cell Biochem. 53, 572–582.
[18] Dhein, J., Walczak, H., Baumler, C., Debatin, K.M. and Krammer, P.H. (1995) Nature 373, 438–441.
[19] Long, L.H., Clement, M.V. and Halliwell, B. (2000) Biochem. Biophys. Res. Commun. 273, 50–53.
[20] Clement, M.V., Ramalingam, J., Long, L.H. and Halliwell, B. (2001) Antioxid. Redox Signal. 3, 157–163.
[21] Tietze, F. (1969) Anal. Biochem. 27, 502–522.
[22] Yonehara, S., Ishii, A. and Yonehara, M. (1989) J. Exp. Med. 169, 1747–1756.
[23] Zamzami, N., Marchetti, P., Castedo, M., Zanin, C., Vayssière, J.L., Petit, P.X. and Kroemer, G. (1995) J. Exp. Med. 181, 1661–1672.
[24] Vermes, I., Haanen, C., Steffens-Nakken, H. and Reutelingsperger, C. (1995) J. Immunol. Methods 184, 39–51.
[25] Jovanovic, S.V. and Simic, M.G. (1985) Life Chem. Rep. 3, 124–130.
[26] Vijayalaxmi, S., Reiter, R.J. and Meltz, M.L. (1995) Mutat. Res. 346, 23–31.
[27] Gilad, E., Wong, H.R., Zingarelli, B., Virág, L., O'Connor, M., Salzman, A.L. and Szabo, C. (1998) FASEB J. 12, 685–693.
[28] Gilad, E., Cuzzocrea, S., Zingarelli, B., Salzman, A.L. and Szabo, C. (1997) Life Sci. 60, 169–174.
[29] Barsacchi, R., Kusmic, C., Damiani, E., Carloni, P., Greci, L. and Donato, L. (1998) Free Radic. Biol. Med. 24, 1187–1192.
[30] Medina-Navarro, R., Duran-Reyes, G. and Hicks, J.J. (1999) Endocr. Res. 25, 263–280.
[31] Baeurle, P.A. and Henkel, T. (1994) Annu. Rev. Immunol. 12, 141–179.
[32] Ginn-Pease, M.E. and Whisler, R.L. (1998) Free Radic. Biol. Med. 25, 346–361.
[33] Baysal, E., Sullivan, S.G. and Stern, A. (1989) Int. J. Biochem. 21, 1109–1113.
[34] Laudicina, D.C. and Marnett, L.J. (1990) Arch. Biochem. Biophys. 278, 73–80.
[35] Guilivi, C. and Cadenas, E. (1993) FEBS Lett. 332, 287–290.
[36] Mendiratta, S., Qu, Z. and May, J.M. (1998) Biochim. Biophys. Acta 1380, 389–395.
[37] Halliwell, B. (1996) Free Radic. Res. 25, 439–454.
[38] Podmore, I.D., Griffiths, H.R., Herbert, K.E., Mistry, N. and Lunec, J. (1998) Nature 392, 559.
[39] Los, M., Khazaie, K., Schulze-Osthoff, K., Baeuerle, P.A., Schirrmacher, V. and Chlichlia, K. (1998) J. Immunol. 161, 3050–3055.
[40] Delneste, Y., Jeannin, P., Sebille, E., Aubry, J.P. and Bonnefoy, J.Y. (1996) Eur. J. Immunol. 26, 2981–2988.
[41] Suhara, T., Fukuo, K., Sugimoto, T., Morimoto, S., Nakahashi, T., Hata, S., Shimizu, M. and Ogihara, T. (1998) J. Immunol. 160, 4042–4047.
[42] Iacovitti, L., Stull, N.D. and Johnston, K. (1997) Brain Res. 768, 317–326.
[43] Lezoualc'h, F., Sparapani, M. and Behl, C. (1998) J. Pineal. Res. 24, 168–178.
[44] Skaper, S.D., Anacona, B., Facci, L., Franceschini, D. and Giusti, P. (1998) FASEB J. 12, 725–731.
[45] Post, A., Holsboer, F. and Behl, C. (1998) J. Neurosci. 18, 8236–8246.
[46] Harms, C., Lautenschlager, M., Bergk, A., Freyer, D., Weih, M., Dirnagl, U., Weber, J.R. and Hörtnagl, H. (2000) FASEB J. 14, 1814–1824.
[47] Eck, K.M., Yuan, L., Duffy, L., Ram, P.T., Ayettey, S., Chen, I., Cohn, C.S., Reed, J.C. and Hill, S.M. (1998) Br. J. Cancer 77, 2129–2137.
[48] Melen-Mucha, G., Winczyk, K. and Pawlikowski, M. (1998) Anticancer Res. 18, 3615–3619.