CADMIUM AND SOFT LASER RADIATION EFFECTS ON HUMAN T CELLS VIABILITY AND DEATH STYLE CHOICES

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Abstract. We investigated the effects of low power 830 nm near-infrared laser light and of the highly toxic environmental pollutant cadmium (Cd^{++}) on cultured human peripheral blood lymphocytes and acute leukemia Jurkat T cells viability, proliferation and preferred death form. Our data indicate that Cd^{++} induces a decrease in viability and survival / proliferation of both human peripheral blood mononuclear cells (PBMC) and of Jurkat cells in a concentration and exposure time dependent manner. The effects are more substantial in serum starvation or growth factor lack caused stress conditions. Cd^{++} induces either apoptosis or necrosis in human T cells depending on the cadmium concentration, duration of exposure, and cells microenvironment. At low concentrations of cadmium, exposed cells die exclusively / prevailingly by apoptosis, while at increasing cadmium concentrations demise occurs both by apoptosis and necrosis, necrotic death becoming dominant at high concentrations of cadmium in stress conditions. The 830 nm laser light decreases PBMC viability in growth factor lack induced stress conditions, and impedes cell death in the presence of growth factors. Low level near infrared laser irradiation enhances cadmium effects in lack of growth factors, but offers some protection to human PBMC in the presence of growth factors.

Key words: AlGaInP/GaAs laser, heavy metal pollution, apoptosis, necrosis.

INTRODUCTION

The non-essential heavy metal cadmium (Cd^{++}) is a widespread industrial and environmental pollutant. Since Cd^{++} is not degraded in the environment, the risk of human exposure is increasing constantly, and due to its very long biological half-life (10–30 years) it almost irreversibly accumulates in exposed humans' organs, causing toxicity to lungs, liver, kidneys, pancreas, testis, placenta, bone, and brain [31]. This highly toxic heavy metal, classified as a human carcinogen [43], identified as endocrine disruptor [53], was also shown to damage the immune

Received February 2008.

ROMANIAN J. BIOPHYS., Vol. 18, No. 3, P. 179–193, BUCHAREST, 2008

system, the cardiovascular system, and to affect the nervous system inducing neurological disorders [10, 31, 42]. In spite of the sustained efforts, and the remarkable number of well-designed high-class scientific investigations aiming to reveal early and late effects of varying levels of cadmium exposure in various conditions [1–3, 6–7, 10, 26–29, 31, 33–35, 42–43, 46, 53–54], understanding of the molecular / cellular mechanisms involved in cadmium tissue toxicity is yet poor.

Low Level Laser Therapy (LLLT) proved to be very efficient in relieving pain, reducing inflammation or toxicant / mechanical injury caused, or after-stroke neurological deficits, and improving wound healing [9, 11, 36, 44–45, 48–50, 52], is nowadays used as part of physiotherapy in most countries. However, in spite of impressive progress made in revealing molecular and cellular mechanisms involved in LLLT beneficial effects [4–5, 8, 13–22, 24–25, 30, 32, 37–41, 47, 51] opening new perspectives in its applications, many details of basic processes mediating the photosignal transduction and amplification cascade are yet insufficiently elucidated.

The aim of the present work was to contribute new data concerning Cd^{++} effects on viability, survival / apoptosis induction in normal and leukemic human T cells, as well as the influence of low power infrared laser irradiation on these parameters in normal and toxicant presence induced stress conditions. Investigating the impact of various concentrations of Cd^{++} and of various doses of 830 nm laser light on cell death rates and weight of different death forms, we report dose and exposure time dependent effects in peripheral blood lymphocytes and in leukemic Jurkat cells, as follows: (i) apoptosis was the preferred death form of both peripheral lymphocytes and of leukemia lymphoblasts at low doses and short exposure periods; (ii) Jurkat leukemia lymphoblasts occurred more vulnerable as compared to peripheral blood lymphocytes; (iii) Jurkat cells susceptibility to Cd^{++} deleterious effects appeared even greater in serum starvation caused stress conditions; (iv) increasingly significant necrosis occurred at higher levels and lengths of Cd^{++} exposure of Jurkat cells, more remarkable in serum starvation caused stress conditions; (v) the 830 nm near-infrared laser light enhanced cadmium toxicity in human peripheral lymphocytes cultured in media without growth factors, while the same irradiation doses offered protection in the presence of growth factors.

MATERIALS AND METHODS

Chemicals, supplements and staining kits: Sterile DMSO, the anticoagulant solution phosphate-citrate-dextrose, D-glucose, and standard RPMI 1640 culture medium were purchased from SIGMA CHEMICAL Co. (R6504), Dulbecco's Phosphate buffered Saline (PBS) 10× from BIOCHROME, while the supplements (FCS, fetal calf serum, EU tested, and antibiotics/glutamine) from GIBCO/INVITROGEN. The growth factors (IL-2, IL-4 and GMCSF) were from

Santa Cruz Co., the colorant Trypan Blue was from SIGMA (T8154, GM 960,81, solution 0.4% in 0.81% NaCl and 0.06% Na_2HPO_4), the fluorescent nuclear stains Hoechst 3342 (Hoe) ($\lambda_{ex/emm}$: 347/483 nm), and Propidium Iodide (PI) ($\lambda_{ex/emm}$: 538/617 nm) from SIGMA or Invitrogen Molecular Probes, while all other chemicals were of the best research grade available.

Stock solutions: The fluorophor Hoe was solved in sterile water (10 mg/mL), while PI in PBS (1 mg/mL). Stock solutions of $CdCl_2$ were prepared in sterile water or in PBS, pH 7 (50 mM and 10 mM).

Culture media: The standard RPMI 1640 medium was supplemented with 2 g/L sodium bicarbonate, 10% heat inactivated fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, and the pH was adjusted to 7.2 (complete medium, M). The serum deprived medium was prepared in the same way without adding FCS (M0 – M without FCS). Other modified culture media were prepared from the complete medium FCS (M), adding growth factors: medium with growth factors (M-GF – M with 1 ng/mL IL2, 5 ng/mL IL4, and 10 ng/mL GMCSF). Cadmium-containing media were prepared from M0 (M0XCd – M containing XmM $CdCl_2$), from M (MXCd), or from M-GF (M-GFXCd).

Cells: Human Leukemia Jurkat T lymphoblasts were from ATCC (TIB-152). Human Peripheral Blood Mononuclear Cells (PBMC) were obtained from venous blood collected in a PCD buffer, of drug-free healthy volunteer donors. All donors signed an informed consent, and protocols were approved by the University Ethics Committee. PBMC were separated from the venous blood by the standard Ficoll-Hypaque density gradient centrifugation, obtaining ~ 15% adherent cells constituted of monocytes, and ~ 65–70% CD3+ T cells. Non-adherent cells constituted mainly of human peripheral blood lymphocytes were occasionally separated from monocytes, by 2–4 h sedimentation allowance of adherent cells, and careful supernatant removal.

Cell culture: The human acute leukemia T Jurkat cells were cultured in flasks or Petri dishes of variable surfaces, in a 5% CO_2 humidified (80%) atmosphere at 37 °C, in 10% FCS containing complete RPMI medium (M), in serum deprived medium (M0) or in cadmium containing media (M0XCd and MXCd), and passaged every 2/3 days through dilution of cell suspensions with fresh media to a concentration of 5–6 × 10^5 cells/mL. Human PBMCs were cultured in the same standard conditions (37 ºC, 80% humidity, 5% CO_2) in complete medium, supplemented (M-GF) or not (M) with growth factors, and containing (MXCd or M-GF-XCd) or not, various concentrations of $CdCl_2$.

Cadmium exposure: Cells were cultured in various cadmium containing media (M0XCd, MXCd or M-GF-XCd, X varying in the range 0.1–20, indicating $CdCl_2$ concentrations of 0.1–20 μM) for various time periods (20–120 h).

The radiation source was an AlGaInP/GaAs based semiconductor laser used in the medical practice, Sony SLD202-D3, with continuous wave output, having emission wavelength of 830 nm, nominal power of 50 mW, elliptical beam size

2.5 mm × 7.0 mm with speckle area of 13.7 mm^2, divergence 5º and polarization ratio 100:1, with an emitted laser power density in the expander-increased speckle area of 100 mW/cm^2.

Sample irradiation regimes and laser irradiation doses: Sample irradiation was performed in the laminar flow, with sources placed at 10 cm height from the upper surface of cell suspensions, in 2 consecutive positions as to cover with the expanded laser speckle the whole surface of the suspension-containing Petri dishes rotating at a speed of 0.5 s^{-1}. Duration of treatment varied between 0–600 s, giving single incident doses equivalent with $(1\div5)\times10^{12}$ photons/cell or ~ (0.2–1.5) μJ/cell. Irradiation regimes of once or twice per day, or every second day with these single doses gave total irradiation doses of ~ (1–15) μJ/cell.

Trypan Blue exclusion method for cell viability and proliferation assessment: After 16–22 h of serum starvation, cells in metabolic quiescence were resuspended in various media at a density of $0.8–0.9\times10^6$ cells/mL, distributed in 3.5 cm diameter Petri dishes, and further cultured for 18–96 h. At indicated time points cells in aliquots of 50 μL were stained with the Trypan Blue colorant (final concentration ≤ 0.2%), visualized using a Zeiss Axiovert 25CFL or 40CFL inverted microscope, and counted in a Buerker-Tuerk hemocytometer. Alternately, micrographs were taken using a Sony 4MP digital camera. Dead cells having permeable plasma membranes are stained by Trypan Blue, live cells with intact plasma membranes exclude this colorant. Counting both live (Trypan Blue excluding, TB^-), and dead (TB^+) cells, the percentage cell viability (ratio of live and total cell numbers):

$$\% \quad \text{viability} = \frac{n_{TB^-}}{n_{TB^-} + n_{TB^+}} \cdot 100 \tag{1}$$

percentage of dead cells (ratio of dead and total cell numbers):

$$\% \quad \text{dead cells} = \frac{n_{TB^+}}{n_{TB^-} + n_{TB^+}} \cdot 100 \tag{2}$$

and/or relative total live cell number (ratio of actual and initial live cell numbers):

$$\text{relative live cell no} = \frac{n_{\text{actual } TB^-}}{n_{\text{initial } TB^-}} \tag{3}$$

were calculated.

Hoescht Staining. Cells grown on tissue culture dishes distributed in 12/24 well plates were incubated with Hoe (2 μg/mL) for 5 min at 37 °C. The cells were then washed twice with phosphate-buffered saline and examined immediately or fixed with 2% paraformaldehyde for 10 min at 4 °C, and visualized within 1 week

by using a Zeiss Axiovert25 CFL and 40CFL inverted microscope with appropriate excitation/emission filters (Filter Set Zeiss 488002-0000).

Propidium Iodide Staining. Cells grown on tissue culture dishes distributed in 12/24 well plates were incubated with PI (1 μg/mL) for 5 min at 37 °C, washed twice with cold PBS, resuspended in PBS, and examined immediately using a Zeiss Axiovert 25CFL or 40CFL inverted microscope with appropriate excitation/emission filters (Filter Set Zeiss 488014-0000). Alternately cells were fixed with 2% paraformaldehyde for 10 min at 4 °C. Fixed cells were visualized within 1 week.

Fluorescence microscopy for assessment of apoptotic and necrotic cell rates: Hoe and PI stained cells were examined using a Zeiss Axiovert 25CFL and 40CFL inverted microscope with 20× or PlanFluar 40× objectives, and appropriate excitation/emission filters. Routinely cells from five random microscopic fields of view at ×200 magnifications were scrutinized. Alternately, images were captured with a Sony 4MP digital camera, and processed later. By morphologic inspection cells were identified as having normal, fragmented, or condensed nuclei. Hoe stained cells with fragmented or condensed nuclei were classified as apoptotic (*A*, Hoe+Ap.Morphol.). PI stained cells were classified as late apoptotic and necrotic (*LA*+*N*, PI+). PI stained cells with fragmented or condensed nuclei were classified as late apoptotic (*LA*, PI+Ap.Morphol.), while those with normal nuclei as necrotic (*N*, PI+Non-Ap.Morphol.). Average total/early/late apoptotic (*A*/*EA*/*LA*) and necrotic cell rates (*N*), and average total dead cells proportion (*A*+*N*), expressed as % of total or of total dead cell numbers were calculated as follows:

$$\%\, A = \frac{n_{\mathrm{Hoe^+Apopt.Morphol.}}}{n_{\mathrm{Hoe^+}}} \cdot 100 \text{ or } \%\, A = \frac{n_{\mathrm{Hoe^+Apopt.Morphol.}}}{n_{\mathrm{total\ dead}}} \cdot 100 \tag{4}$$

$$\%\, N = \frac{n_{\mathrm{PI^+Non\text{-}Apopt.Morphol}}}{n_{\mathrm{total\ cells}}} \cdot 100 \text{ or } \%\, N = \frac{n_{\mathrm{PI^+Non\text{-}Apopt.Morphol}}}{n_{\mathrm{total\ dead}}} \cdot 100 \tag{5}$$

$$\%\, LA = \frac{n_{\mathrm{PI^+Apopt.Morphol}}}{n_{\mathrm{total\ cells}}} \cdot 100 \tag{6}$$

$$\%\, EA = \frac{n_{\mathrm{Hoe^+Apopt.Morphol}} - n_{\mathrm{PI^+Apopt.Morphol}}}{n_{\mathrm{total\ cells}}} \cdot 100 \tag{7}$$

where $n_{\mathrm{totaldead}} = n_{\mathrm{Hoe+Apopt.Morphol.}} + n_{\mathrm{PI+Non\text{-}Apopt.Morphol,}}$, while $n_{\mathrm{totalcells}}$ was counted in phase contrast or bright field.

Statistical Analysis: Viabilities and percentage and relative cell subpopulation sizes were obtained as means calculated from at least 3 independent assessments

(S.D. ≤ 15%). Unpaired analysis of data series obtained by measurements made on cells various time periods after their transfer in various modified media, irradiated and/or not irradiated, was performed by Student's t-test (two-tailed). *P*-values less than 0.05 were regarded as indicating statistical significance.

RESULTS

Experiments were performed to assess human PBMC viability and proliferation, and changes occurring in these parameters in cadmium-treated and/or irradiated samples in various conditions. Counting live and dead cells in trypan blue stained samples in a hemocytometer, following 18–54 h exposure to a dose of 2–20 μM Cd^{++}, the heavy metal-induced cytotoxicity and cell growth inhibition was evident, as well as the sensitive modulation by soft laser irradiation of the observed effects. Figure 1 illustrates the Cd^{++} concentration and exposure length dependent increase in the death rate of exposed cells.

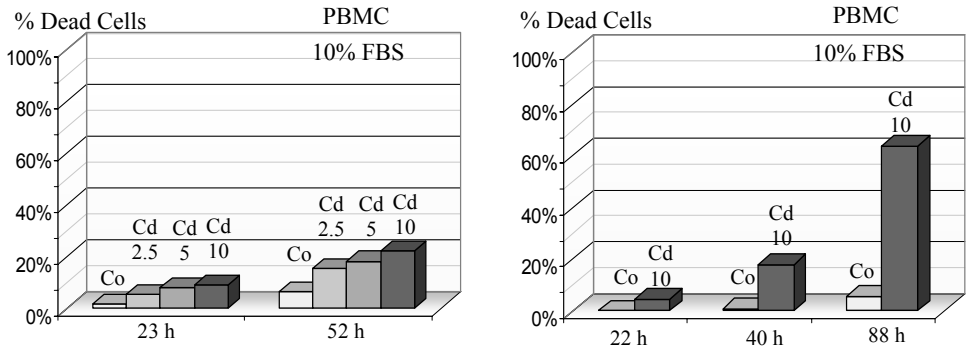

Fig. 1. Dose and exposure time dependent cadmium effects on dead cells rate in human PBMC populations; % of dead cells determined by the trypan exclusion method, counting trypan excluding (TB^-) and trypan not excluding (TB^+) cells in a Neubauer-Tuerk hemocytometer, using a Zeiss Axiovert 25CFL or 40CFL microscope, various time lags after cells resuspension in media containing different Cd^{++} concentrations. Represented entities were calculated as averages of values obtained in 3 independent measurements; S.D. ≤ 15%.

Figures 2 and 3 illustrate the changes occurring in human PBMC viability and proliferation/survival rate following their exposure to deleterious effects of 10 μM Cd^{++}. Obviously longer exposure times result in increased damage, and cadmium toxicity is higher in growth factor deprived media, as compared to that observed in the presence of growth factors.

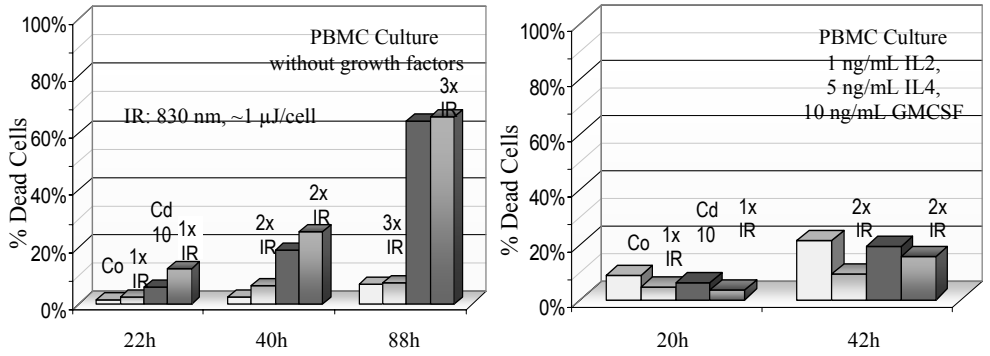

Fig. 2. Photobiomodulation of microenvironment and exposure time dependent cadmium effects on dead cells rates in human PBMC populations; % of dead cells determined by the trypan blue exclusion method, counting trypan excluding (TB^-) and trypan not excluding (TB^+) cells in a Neubauer-Tuerk hemocytometer, using a Zeiss Axiovert 25CFL or 40CFL microscope, various time lags after cells resuspension in media without/with growth factors containing 10 μM Cd^{++} (M10Cd and M-GF-10Cd). Not irradiated controls (Co) and cell suspensions irradiated with 1, 2 or 3 single doses of 1 μJ/cell of 830 nm near-infrared laser light (1×IR, 2×IR, 3×IR). Represented entities were calculated as averages of values obtained in 3 independent measurements; S.D. ≤ 15%.

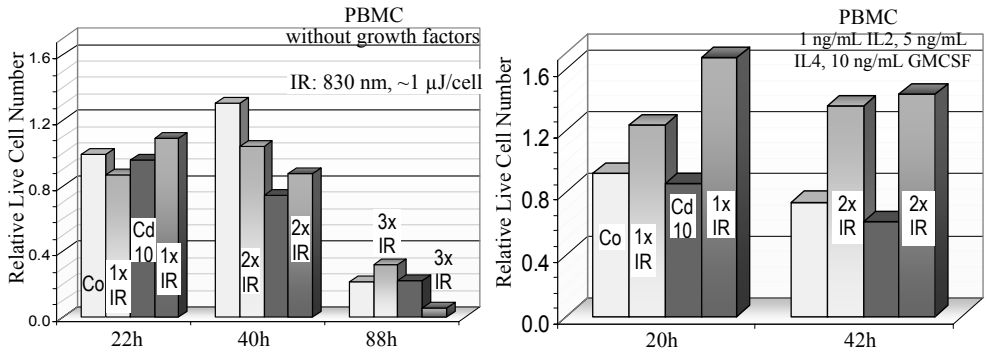

Fig. 3. Photobiomodulation of microenvironment and exposure time dependent cadmium effects on human PBMC proliferation/survival; Relative live cell numbers determined by the trypan blue exclusion method, counting trypan excluding (TB^-) and trypan not excluding (TB^+) cells in a Neubauer-Tuerk hemocytometer, using a Zeiss Axiovert 25CFL or 40CFL microscope, various time lags after cells resuspension in media without/with growth factors containing 10 μM Cd^{++} (M10Cd and M-GF-10Cd). Not irradiated controls (Co and Cd10) and cell suspensions irradiated with 1, 2 or 3 single doses of 1 μJ/cell of 830 nm near-infrared laser light (1×IR, 2×IR, 3×IR).

Irradiation with the 830 nm laser light increases the dead cells rates in the absence of growth factors, and decreases them in the presence of growth factors both in control and in cadmium exposed human PBMC. Decrease in relative live cell numbers is prevented, and control and cadmium exposed human PBMC survival/proliferation is clearly promoted by doses of 1–2 μJ/cell of the 830 nm near infrared laser light in the presence of growth factors.

Human leukemia T lymphoblasts (Jurkat) are more susceptible to cadmium toxicity, as compared to human PBMC. Harmful effects are more substantial in media deprived of FCS (FBS). Figure 4 illustrates the Cd^{++} concentration and exposure length dependent increase in the death rate of exposed Jurkat cells.

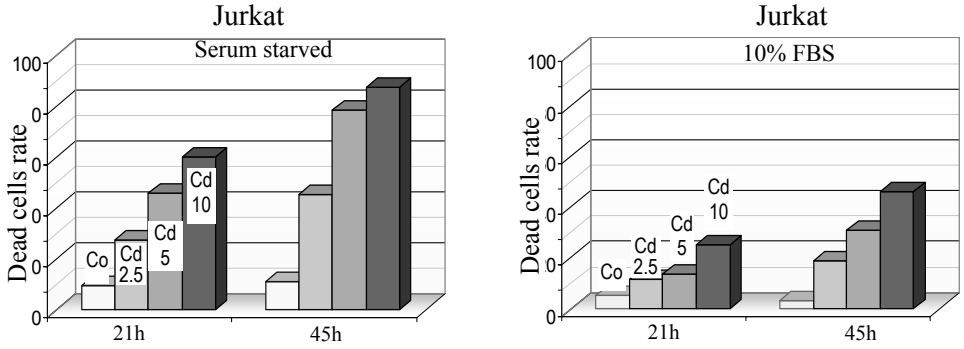

Fig. 4. Dose and exposure time dependent cadmium effects on dead cells rate in Jurkat cell populations, observed in FCS deprived and in FCS supplemented media; % of dead cells determined by the trypan exclusion method, counting trypan excluding (TB^-) and trypan not excluding (TB^+) cells in a Neubauer-Tuerk hemocytometer, using a Zeiss Axiovert 25CFL or 40CFL microscope, various time lags after cells resuspension in normal and serum deprived media containing different Cd^{++} concentrations. Represented entities were calculated as averages of values obtained in 3 independent measurements; S.D. ≤ 15%.

Figures 5–8 and Tables 1–4 illustrate the cadmium exposed Jurkat cells death-style choices in serum supplemented and serum deprived media at increasing levels and lengths of exposure.

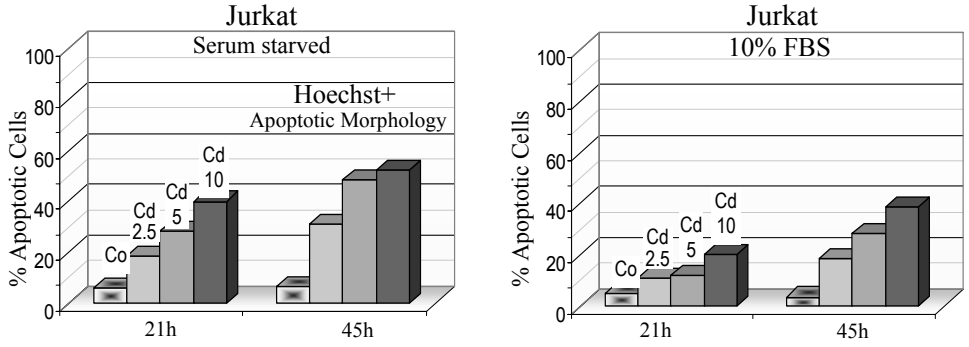

Fig. 5. Dose and exposure time dependent cadmium effects on apoptotic cells rate in Jurkat cell populations, observed in FCS deprived and in FCS supplemented media: % of apoptotic cells determined by fluorescence microscopy, counting Hoechst stained Jurkat cells – total and with apoptotic nuclear morphology (Hoe^+Apopt.Morphol.) – in 5 random microscopic fields, using a Zeiss Axiovert 25CFL or 40CFL microscope (magnification x200), various time lags after cells resuspension in normal and serum deprived media containing different Cd^{++} concentrations. Represented entities were calculated as averages of values obtained in 3 independent measurements; S.D. ≤ 15%.

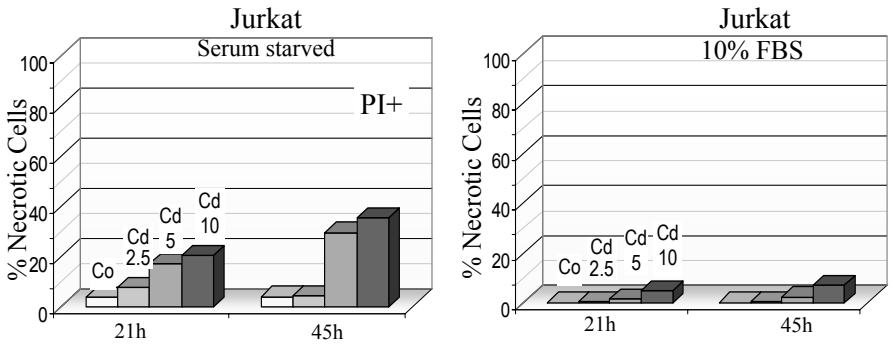

Fig. 6. Dose and exposure time dependent cadmium effects on necrotic cells rate in Jurkat cell populations, observed in FCS deprived and in FCS supplemented media: % of necrotic cells determined by fluorescence microscopy, counting Propidium Iodide stained Jurkat cells with non-apoptotic nuclear morphology (PI+Non-Apopt.Morphol.) in 5 random microscopic fields, and total cell numbers in 5 random bright fields, using a Zeiss Axiovert 25CFL or 40CFL microscope (magnification ×200), various time lags after cells resuspension in normal and serum deprived media containing different Cd^{++} concentrations. Represented entities were calculated as averages of values obtained in 3 independent measurements; S.D. ≤ 15%.

In serum containing media cadmium exposed Jurkat cells die by apoptosis. In serum deprived media at high levels and long exposure periods apoptotic cell death is accelerated (Fig. 7–8), and necrosis becomes an important death form (Fig. 6, Tables 1–4).

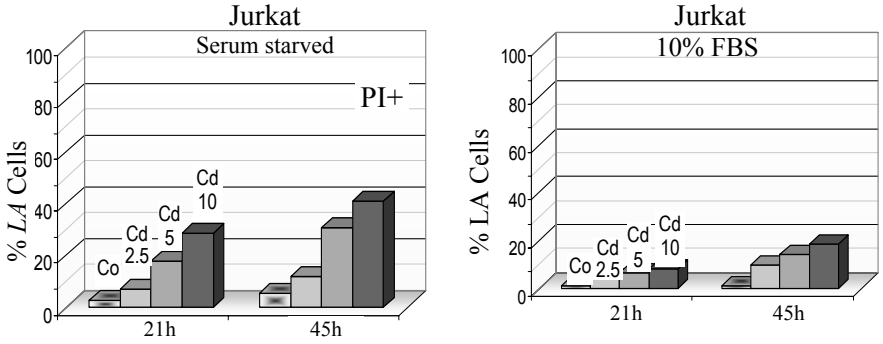

Fig. 7. Dose and exposure time dependent cadmium effects on late apoptotic cells rate in Jurkat cell populations, observed in FCS deprived and in FCS supplemented media; % of late apoptotic cells determined by fluorescence microscopy, counting Propidium Iodide stained Jurkat cells with apoptotic nuclear morphology (PI+Apopt.Morphol.) in 5 random microscopic fields, and total cell numbers in 5 random bright fields, using a Zeiss Axiovert 25CFL or 40CFL microscope (magnification ×200), various time lags after cells resuspension in normal and serum deprived media containing different Cd^{++} concentrations.

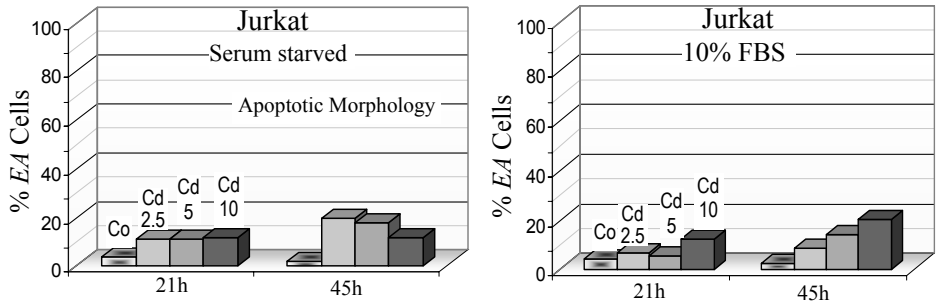

Fig. 8. Dose and exposure time dependent cadmium effects on early apoptotic cells rate in Jurkat cell populations, observed in FCS deprived and in FCS supplemented media: % of early apoptotic cells determined by fluorescence microscopy, counting Hoechst stained and and Propidium Iodide stained Jurkat cells with apoptotic nuclear morphology (Hoe+Apopt.Morphol. - PI+Apopt.Morphol.) in 5 random microscopic fields, and total cell numbers in 5 random bright fields, using a Zeiss Axiovert 25CFL or 40CFL microscope (magnification ×200), various time lags after cells resuspension in normal and serum deprived media containing different Cd^{++} concentrations. Represented entities were calculated as averages of values obtained in 3 independent measurements; S.D. ≤ 15%.

Table 1

Death style choices of serum starved Jurkat cells exposed 21 h to deleterious effects of 10 μM Cd^{++}. Live, dead, total/early/late apoptotic, and necrotic cell rates determined by conventional and fluorescence microscopic inspection of TB, Hoe, and PI stained cells, and expressed as percentages of total (live and dead) and total dead cell numbers. Represented entities were calculated as averages of values obtained in 3 independent measurements; S.D. ≤ 15%

Sample	Live[1] % total cell number	Dead[2] % total cell number	Apoptotic[3] % dead cell number	Necrotic[4] % dead cell number	Early Apoptotic[5] % apoptotic cell number	Late Apoptotic[6] % apoptotic cell number
Control	90.32	9.88	63.98	38.06	55.06	44.94
Cd2.5	72.37	26.83	68.57	28.47	60.87	39.13
Cd5	54.13	45.79	62.51	37.35	37.76	62.24
Cd10	39.69	60.32	66.65	33.36	28.86	71.14

[1]TB data; [2]Average TB, Hoe & PI Data; [3]Hoe & Nuclear Morphology Data; [4]PI & Nuclear Morphology Data; [5]Hoe, PI & Nuclear Morphology Data; [6]PI & Nuclear Morphology Data

Table 2

Death style choices of serum starved Jurkat cells exposed 45 h to deleterious effects of 10 μM Cd^{++}. Live, dead, total/early/late apoptotic, and necrotic cell rates determined by conventional and fluorescence microscopic inspection of TB, Hoe, and PI stained cells, and expressed as percentages of total (live and dead) and total dead cell numbers. Represented entities were calculated as averages of values obtained in 3 independent measurements; S.D. ≤ 15%

Sample	Live[1] % total cell number	Dead[2] % total cell number	Apoptotic[3] % dead cell number	Necrotic[4] % dead cell number	Early Apoptotic[5] % apoptotic cell number	Late Apoptotic[6] % apoptotic cell number
Control	88.78	11.01	62.13	35.97	23.39	76.61
Cd2.5	54.61	40.54	77.46	10.56	61.91	38.09

Table 2 (continued)

Cd5	21.41	78.31	62.01	37.53	36.88	63.12
Cd10	12.15	87.91	59.61	40.45	22.02	77.98

[1–6] Significance is the same as in Table 1.

Table 3

Death style choices of Jurkat cells exposed 21 h to deleterious effects of 10 μM Cd^{++} in 10% FCS containing medium. Live, dead, total/early/late apoptotic, and necrotic cell rates determined by conventional and fluorescence microscopic inspection of TB, Hoe, and PI stained cells, and expressed as percentages of total (live and dead) and total dead cell numbers. Represented entities were calculated as averages of values obtained in 3 independent measurements; S.D. ≤ 15%

Sample	Live[1] % total cell number	Dead[2] % total cell number	Apoptotic[3] % dead cell number	Necrotic[4] % dead cell number	Early Apoptotic[5] % apoptotic cell number	Late Apoptotic[6] % apoptotic cell number
Control	94.80	5.24	100	0.76	75.57	24.43
Cd2.5	88.64	11.32	95.41	4.24	61.11	38.89
Cd5	86.54	13.51	89.71	10.66	43.89	56.11
Cd10	74.66	25.28	80.78	18.99	58.89	41.14

[1–6] Significance is the same as in Table 1.

Table 4

Death style choices of Jurkat cells exposed 45 h to deleterious effects of 10 μM Cd^{++} in 10% FCS containing medium. Live, dead, total/early/late apoptotic, and necrotic cell rates determined by conventional and fluorescence microscopic inspection of TB, Hoe, and PI stained cells, and expressed as percentages of total (live and dead) and total dead cell numbers. Represented entities were calculated as averages of values obtained in 3 independent measurements; S.D. ≤ 15%

Sample	Live[1] % total cell number	Dead[2] % total cell number	Apoptotic[3] % dead cell number	Necrotic[4] % dead cell number	Early Apoptotic[5] % apoptotic cell number	Late Apoptotic[6] % apoptotic cell number
Control	96.84	3.42	100.00	1.17	70.33	29.67
Cd2.5	81.24	18.78	97.98	2.13	46.09	53.91
Cd5	68.94	30.95	92.42	7.21	49.58	50.42
Cd10	54.12	45.97	84.76	15.42	52.18	47.82

[1–6] Significance is the same as in Table 1.

DISCUSSION AND CONCLUSIONS

Given the need to find ways to prevent/reverse heavy metals tissue toxicity, cellular effects of the highly toxic xenobiotic Cd^{++} are intensely studied. The so-far revealed molecular mechanisms involved in cadmium toxicity are enhancement of the free radical production and interference with the cellular defense mechanisms against oxidation [31, 34, 53], modulation of cytokine production in immune cells [10], up-regulation of gene-expression, especially of those related to inflammation,

survival and apoptosis [43], ceramide production, sequential calpain and caspase activation, mitochondrial damage with release of cytochrome C, apoptosis inducing factor (AIF) and ATP in later stages [31]. Apoptosis induction was demonstrated in various cell-types [2, 6–7, 26–29, 31, 33–34, 46, 54], and it is generally accepted that the cellular processes responsible for the development of cadmium toxicity culminate in triggering of cell death by either apoptosis or necrosis [6, 31]. However, programmed cell death also plays an important beneficial role in homeostasis of multicellular organisms, so that impaired apoptotic rates may be as damaging as increased ones [12, 23], and induction of apoptosis resistance and enhancement of cell proliferation were shown as underlying Cd^{++} carcinogenic effects [1]. Thus far, the dependence on level, length, and conditions of exposure of cadmium effects – pro- or anti-apoptotic, apoptosis or necrosis promoting – in different cell-types is poorly understood.

Promotion of cell proliferation [4–5, 25, 37, 47], *de novo* protein synthesis [40], survival and cell cycle entry [41], reversal of toxicity caused apoptosis induction [8, 30, 51] by low power long wavelength laser light, was repeatedly reported, and we also described significant modulation by the low power 680 nm far-red and 830 nm near-infrared laser irradiation of quercetin caused apoptosis induction in human leukemic cells *in vitro* [38]. Though disclosure of the exact dependence of the effects on fluence rate, frequency of irradiation, and individual and total doses await further thorough investigations, cell type and state and exposure conditions dependence of laser effects is apparent.

Investigating impact of low, micro-molar, concentrations of Cd^{++} and of low doses of 830 nm laser light on cell death rates and death-style choices, here we report dose and exposure time dependent effects in both peripheral blood lymphocytes and in leukemia Jurkat cells. Our data indicate apoptosis as preferred death form at low doses and short exposure periods, document increasingly significant necrosis occurrence at higher levels and lengths of exposure, confirm serum offered protection and cell type dependence of the induced changes, and point out significant microenvironment dependent photobiomodulation of Cd^{++} effects in human T cells.

Acknowledgments. Partial financial support of the Ministry of Education and Research of Romania (Grant CNCSIS 924/2006 and Grant CEEX 60/2005) is gratefully acknowledged.

REFERENCES

1. ARRIAZU, R., J. M. POZUELO, N. HENRIQUES-GIL, T. PERUCHO, R. MARTÍN, R. RODRÍGUEZ, L. SANTAMARÍA, Immunohistochemical study of cell proliferation, Bcl-2, p53, and Caspase-3 expression on preneoplastic changes induced by cadmium and zinc chloride in the ventral rat prostate, J. Histochem. Cytochem., 2006, **54**, 981–990.
2. BAGCHI, D., S.S. JOSHI, M. BAGCHI, J. BALMOORI, E.J. BENNER, C.A. KUSZYNSKI, S.J. STOHS, Cadmium- and chromium-induced oxidative stress, DNA damage, and apoptotic cell death in cultured human chronic myelogenous leukemic K562 cells, promyelocytic leukemic

HL-60 cells, and normal human peripheral blood mononuclear cells, *J. Biochem. Mol. Toxicol*., 2000, **14**, 33–41.
3. BERTIN, G., D. AVERBECK, Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences, *Biochimie,* 2006, **88**, 1549–1559.
4. BYRNES, K.R., *et al*., Cellular invasion following spinal cord lesion and low power laser irradiation, *Lasers Surg. Med*., 2002, **S14**, 11–16.
5. CHENG, L., T.C. LIU, J.-Q. CHI, Y. LI, H. JIN, Photobiomodulation on the proliferation and collagen synthesis of normal human skin fibroblast cells, *Proceedings of the SPIE*, 2006, **6026**, 63–72.
6. CIARIMBOLI, G., Unraveling the ceramide-calpain-caspase connection in cadmium-induced apoptosis: a novel role for ceramides as activators of calpains. Focus on "Cadmium-induced ceramide formation triggers calpain-dependent apoptosis in cultured kidney proximal tubule cells", *Am. J. Physiol. Cell. Physiol.,* 2007, **293,** C837–C838.
7. COUTANT, A., J. LEBEAU, N. BIDON-WAGNER, C. LEVALOIS, B. LECTARD AND S. CHEVILLARD, Cadmium-induced apoptosis in lymphoblastoid cell line: involvement of caspase-dependent and -independent pathways, *Biochimie*, 2006, **88**, 1815–1822.
8. EELLS, J.T., M.M. HENRY, P. SUMMERFELT, M.T.T. WONG-RILEY, E.V. BUCHMANN, M. KANE, N.T. WHELAN, H.T. WHELAN, Therapeutic photobiomodulation for methanol-induced retinal toxicity, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 3439–3444.
9. ENWEMEKA, C.S., J.C. PARKER, D.S. DOWDY, E.E. HARKNESS, L.E. SANFORD, L.D. WOODRUFF, The efficacy of low power lasers in tissue repair and pain control: a meta-analysis study, *Photomed. Laser Surg*., 2004, **22**, 323–329.
10. GUINTO-VILLANUEVA, M. B., S. KOIZUMI, H. JONAI, Cytokine production by human peripheral blood mononuclear cells after exposure to heavy metals, *Journal of Health Science*, 2000, **46**, 358–362.
11. HAWKINS, D., HEIDI ABRAHAMSE, Phototherapy – a treatment modality for wound healing and pain relief, *Afr. J. Biomed. Res.*, 2007, **10**, 99–109.
12. HEIMLICH, G., J. A. CIDLOWSKI, Selective role of intracellular chloride in the regulation of the intrinsic but not extrinsic pathway of apoptosis in Jurkat T-cells, *J. Biol. Chem.*, 2006, **281**, 2232–2241.
13. KARU, T, L.V. PYATIBRAT, S.F. KOLYAKOV, N.I. AFANASYEVA, Absorption measurements of a cell monolayer relevant to phototherapy: Reduction of cytochrome c oxidase under near IR radiation, *J. Photochem. Photobiol. B: Biology*, 2005, **8507**, 23–32.
14. KARU, T., Mechanisms of low-power laser light action on cellular level, In: *Lasers in Medicine and Dentistry,* Z. Simunovic (Ed.), Vitgraf, Rijeka, 2000, pp. 97–125.
15. KARU, T., S.F. KOLYAKOV, Exact action spectra for cellular responses relevant to phototherapy, *Photomed. Laser Surg*., 2005, **23**, 355–361.
16. KARU, T., *Ten Lectures on Basic Science of Laser Phototherapy*, Prima Books AB, Grangesberg, Sweden, 2007.
17. KARU, T.I., Cellular mechanism of low power laser therapy: new questions, In: *Lasers in Medicine and Dentistry*, Vol. 3., Z. Simunovic (Ed.), Vitgraf, Rijeka, 2003, Ch. IV., pp. 79–100.
18. KARU, T.I., L.V. PYATIBRAT, G.S. KALENDO, Photobiological modulation of cell attachment via cytochrome c oxidase, *Photochem. Photobiol. Sci*., 2004, **3**, 211–216.
19. KARU, T.I., L.V. PYATIBRAT, G.S. KALENDO, Studies into the action specifics of a pulsed GaAlAs laser (λ = 820 nm) on a cell culture. I. Reduction of the intracellular ATP concentration: dependence on initial ATP amount, *Lasers Life Sci*., 2001, **9**, 203–210.
20. KARU, T.I., L.V. PYATIBRAT, G.S. KALENDO, Studies into the action specifics of a pulsed GaAlAs laser (λ = 820 nm) on a cell culture. II. Enhancement of the adhesive properties of cellular membranes: dependence on the dark period between pulses, *Lasers Life Sci*., 2001, **9**, 211–217.

21. KARU, T.I., Low power laser therapy, In: *Biomedical Photonics Handbook*, Tuan Vo-Dinh (Ed.), CRC Press, Boca Raton 2003, Ch. 48, pp. 48-1–48-25.
22. KARU, T.I., Low-power laser effects. In: *Lasers in Medicine*, R. Waynant (Ed.), Boca Raton, CRC Press, 2002, pp. 171–209.
23. KHAN, M.T., C. D. BHANUMATHY, Z.T. SCHUG, S.K. JOSEPH, Role of inositol 1,4,5-trisphosphate receptors in apoptosis in DT40 lymphocytes, *J. Biol. Chem*., 2007, **282**, 32983–32990.
24. KOJI, M., A. AKIRA, A.A. TAKASAKI, A. KINOSHITA, C. HAYASHI, S. ODA, I. ISHIKAWA, Periodontal tissue healing following flap surgery using an Er:YAG laser in dogs, *Lasers Surg. Med*., 2006, **38**, 314.
25. KREISLER, M., A.B. CHRISTOFFERS, B. WILLERSHAUSEN, B. D'HOEDT, Effect of low-level GaAlAs laser irradiation on the proliferation rate of human periodontal ligament fibroblasts: an *in vitro* study, *J. Clin. Periodontol*., 2003, **30**, 353.
26. LÅG, M. , S. WESTLY, T. LERSTAD, C. BJØRNSRUD, M. REFSNES, P.E. SCHWARZE, Cadmium-induced apoptosis of primary epithelial lung cells: Involvement of Bax and p53, but not of oxidative stress, *Cell Biol. Toxicol.*, 2002, 18, 1573–6822.
27. LEE, W.-K., M. ABOUHAMED, F. THEVENOD, Caspase-dependent and -independent pathways for cadmium-induced apoptosis in cultured kidney proximal tubule cells, *Am. J. Physiol. Renal. Physiol.*, 2006, **291**, F823–F832.
28. LEE, W-K, B. TORCHALSKI, F. THEVENOD, Cadmium-induced ceramide formation triggers calpain-dependent apoptosis in cultured kidney proximal tubule cells, *Am. J. Physiol. Cell Physiol.*, 2007, **293,** C839–C847.
29. LI, M., T. KONDO, Q.-L. ZHAO, F.-J. LI, K. TANABE, Y. ARAI, Z.-C. ZHOU, M. KASUYA, Apoptosis induced by cadmium in human lymphoma U937 cells through Ca^{2+}-calpain and caspase-mitochondria dependent pathways, *J. Biol. Chem.***,** 2000, **275**, 39702–39709.
30. LIANG, H.L., H.T. WHELAN, J.T. EELLS, H. MENG, E. BUCHMANN, A. LERCH-GAGGL, M. WONG-RILEY, Photobiomodulation partially rescues visual cortical neurons from cyanide-induced apoptosis, *Neurosci.*, 2006, **139**, 639–649.
31. LOPEZ, E., S. FIGUEROA, M.J. OSET-GASQUE, M.P. GONZALEZ, Apoptosis and necrosis: two distinct events induced by cadmium in cortical neurons in culture, *Br. J. Pharmacol.*, 2003, **138**(5), 901–911.
32. LUBART, R, M. EICHLER, R. LAVI, H. FRIEDMAN, A. SHAINBERG, Low-energy laser irradiation promotes cellular redox activity, *Photomed. Laser Surg*., 2005, **23**, 3–9.
33. MAO, W.-P., J.-L. YE, Z.-B. GUAN, J.-M. ZHAO, C. ZHANG, N.-N. ZHANG, P. JIANG, T. TIAN, Cadmium induces apoptosis in human embryonic kidney (HEK) 293 cells by caspase-dependent and -independent pathways acting on mitochondria, *Toxicol. in vitro*, 2007, **21**, 343–354.
34. OH, S.H., S.C. LIM, A rapid and transient ROS generation by cadmium triggers apoptosis via caspase-dependent pathway in HepG2 cells and this is inhibited through N-acetylcysteine-mediated catalase upregulation, *Toxicol. Appl. Pharmacol*., 2006, **212**, 212–223.
35. OH, S.H., J.E CHOI, S.C. LIM, Protection of betulin against cadmium-induced apoptosis in hepatoma cells, *Toxicology*, 2006, **220**, 1–12.
36. ORON, A., U. ORON, J. CHEN, A. EILAM, C. ZHANG, M. SADEH, Y. LAMPL, J. STREETER, L. DETABOADA, M. CHOPP, Low-level laser therapy applied transcranially to rats after induction of stroke significantly reduces long-term neurological deficits, *Stroke*, 2006, **37**, 2620–2624.
37. POURZARANDIAN, A., H. WATANABE, S.M.P.M. RUWANPURA, A. AOKI, I. ISHIKAWA, Effect of low-level Er:YAG laser irradiation on cultured human gingival fibroblasts, *J. Periodont*., 2005, **76**, 187–193.
38. SEREMET, T., M. DUMITRESCU, S. RADESI, GY. KATONA, I. O. DOAGĂ, E. RADU, J. HORVÁTH5, E. TANOS, L. KATONA, E. KATONA, Photobiomodulation of quercetin antiproliferative effects, seen in human acute T leukemic Jurkat cells, *Rom. J. Biophys*., 2007, **17**, 33–43.

39. SHAIK, Z.P., E.K. FIFER, G. NOWAK, Akt activation improves oxidative phosphorylation in renal proximal tubular cells following nephrotoxicant injury, *Am. J. Physiol. Renal Physiol*., 2008, **294**, F423–F432.
40. SHEFER, G., I. BARASH, U. ORON, O. HALEVY, Low-energy laser irradiation enhances de novo protein synthesis via its effects on translation-regulatory proteins in skeletal muscle myoblasts, *Biochim. Biophys. Acta*, 2003, **1593**, 131–139.
41. SHEFER, G., T.A. PARTRIDGE, L. HESLOP, J.G. GROSS, U. ORON, O. HALEVY, Low energy laser irradiation promotes the survival and cell cycle entry of skeletal muscle satellite cells, *J. Cell Science*, 2002, **115**, 1461–1469.
42. SHEN, X., K. LEE, R. KÖNIG, Effects of heavy metal ions on resting and antigen-activated $CD4^+$ T cells, *Toxicology*, 2001, **169,** 67–80.
43. SHIN, H.-J., B.-H. LEE, M.G. YEO, S.-H. OH, J.-D. PARK, K.-K. PARK, J.-H. CHUNG, C.-K. MOON, M.-O. LEE, Induction of orphan nuclear receptor Nur77 gene expression and its role in cadmium-induced apoptosis in lung, *Carcinogenesis*, 2004, **25**, 1467–1475.
44. SIMUNOVIC, Z., (Ed.), Lasers in medicine and dentistry – Part one: Basic science, and up-to-date clinical application of Low Energy-Laser Laser Therapy LLLT, www.lasermedico.ch, ISBN 953-6059-30-4, 2000.
45. SPLINTER, R., *An Introduction to Biomedical Optics*, CRC Press, Boca Raton, 2006.
46. STINSONA, L. J., A. J. DARMONA, L. DAGNINOA, S.J.A. D'SOUZA, Delayed apoptosis post-cadmium injury in renal proximal tubule epithelial cells, Am. J. Nephrol., 2003, **23**, 27–37.
47. TUBY, HANA, LIDYA MALTZ, U. ORON, Low-level laser irradiation (LLLI) promotes proliferation of mesenchymal and cardiac stem cells in culture, *Lasers Surg. Med*., 2007, **39**, 373–378.
48. TUNER, J., L. HODE, Laser therapy – clinical practice and scientific background, Prima Books, Grängesberg, 2002.
49. VO-DINH, T. (Ed.), *Biomedical Photonics Handbook*, CRC Press, Boca Raton, 2003.
50. WILSON, B.C., S. TANEV, V.V. TUCHIN, *Advances in Biophotonics*, IOS Press, 2005.
51. WONG-RILEY, M.T.T., H.L. LIANG, J.T. EELLS, B. CHANCE, M.M. HENRY, E. BUCHAMANN, M. KANE, H.T.WHELAN, Photobiomodulatiom directly benefits primary neurons functionally inactivated by toxins, *J. Biol. Chem*, 2005, **280**, 4761–4771.
52. WOODRUFF, L., J. BOUNKEO, W. BRANNON, K. DAWES, C. BARHAM, D. WADDELL, C. ENWEMEKA, The efficacy of laser therapy in wound repair: A meta-analysis of the literature, *Photomed. Laser Surg*., 2004, **22**, 241–247.
53. YANG, K., L. JULAN, F. RUBIO, A. SHARMA, H. GUAN**.** Cadmium reduces 11β-hydroxysteroid dehydrogenase type 2 activity and expression in human placental trophoblast cells, *Am. J. Physiol. Endocrinol. Metab.*, 2006, **290**, E135–E142.
54. YE, J.-L., W.-P. MAO, A.-L. WU, N.-N. ZHANG, C. ZHANG, Y.-J. YU, L. ZHOU, C.-J. WEI Cadmium-induced apoptosis in human normal liver L-02 cells by acting on mitochondria and regulating Ca^{2+} signals, *Environmental Toxicol. Pharmacol*., 2007, **24**, 45–54.