ELECTROMAGNETIC BIOLOGY AND MEDICINE

http://informahealthcare.com/ebm
ISSN: 1536-8378 (print), 1536-8386 (electronic)

Electromagn Biol Med, Early Online: 1–5
© 2014 Informa Healthcare USA, Inc.
DOI: 10.3109/15368378.2014.919591

informa healthcare

ORIGINAL ARTICLE

Effects of microwave exposure and Gemcitabine treatment on apoptotic activity in Burkitt's lymphoma (Raji) cells

Ayşe G. Canseven[1], Meric Arda Esmekaya[1], Handan Kayhan[2], Mehmet Zahid Tuysuz[2], and Nesrin Seyhan[1]

[1]Department of Biophysics and Division of Hematology and [2]Department of Internal Medicine, Faculty of Medicine, Gazi University, Ankara, Turkey

Abstract

We investigated the effects of 1.8 MHz Global System for Mobile Communications (GSM)-modulated microwave (MW) radiation on apoptotic level and cell viability of Burkitt's lymphoma (Raji) cells with or without Gemcitabine, which exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase). Raji cells were exposed to 1.8 GHz GSM-modulated MW radiation at a specific absorption rate (SAR) of 0.350 W/kg in a CO_2 incubator. The duration of the exposure was 24 h. The amount of apoptotic cells was analyzed using Annexin V-FITC and propidium iodide (PI) staining with flow cytometer. The apoptotic activity of MW exposed Raji cells was increased significantly. In addition, cell viability of exposed samples was significantly decreased. Combined exposure of MW and Gemcitabine increased the amount of apoptotic cells than MW radiation alone. Moreover, viability of MW + Gemcitabine exposed cells was lower than that of cells exposed only to MW. These results demonstrated that MW radiation exposure and Gemcitabine treatment have a synergistic effect on apoptotic activity of Raji cells.

Keywords

Apoptosis, cell viability, non-ionizing, radiofrequency

History

Received 5 January 2014
Accepted 20 April 2014
Published online 5 June 2014

Electromagn Biol Med Downloaded from informahealthcare.com by Gazi Univ. on 06/09/14
For personal use only.

Introductıon

Microwaves (MW) are radiofrequency waves with frequencies between 300 MHz (0.3 GHz) and 300 GHz. MW radiation is a non-ionizing electromagnetic radiation that does not carry enough energy for ionization. It meant that it has not enough energy to remove an electron from atoms or molecules. Cell phones, base stations, radars, MW ovens and satellite stations are main sources of MW fields. The most common exposures to MW are from telecommunication devices. It has been raised a publical concern about safety of MW radiation in daily life. There are many published papers in the literature concerning possible biological effects resulting from exposure to MW radiation.

There are studies reporting an increased risk of some types of tumor from exposure to the radiation emitted from mobile phones. The development of acoustic neuroma and glioma risks were increased between 2.9 and 5.4 times for bilateral and ipsilateral mobile phone using for 10 years (Hardell et al., 2006a,b). Repacholi et al. (1997) showed that lymphoma risk increased 2.4 times in MW exposed mice compared to unexposed animals. It has also been shown that modulated MW radiation may increase the level of ornithine decarboxylase (ODC). ODC is an enzyme that is over-expressed in various types of cancers and associated with promoting tumor growth and angiogenesis (Litovitz et al., 1993, 1997a,b; Nemoto et al., 2002; Penafiel et al., 1997; Yakymenko et al., 2011). The International Agency for Research on Cancer (IARC) has classified mobile phone radiation on the IARC scale as a Group 2B – possibly carcinogenic. Genotoxic effects of MW radiation have also been reported in scientific studies which were performed in different cell types. MW radiation may induce DNA breaks (Lai and Singh, 1995) and increase the frequency of chromosome aberrations (CA), micronuclei (Maes et al., 1993) and Sister Chromatid Exchange (Esmekaya et al., 2011). Most of these studies were performed in lymphocytes. Fesenko et al. (1999) and Veyret et al. (1991) showed that low level of MW radiation may affect immune system.

In this study, we researched the effects of an 1.8 MHz GSM-modulated MW radiation on apoptotic activity and cell proliferation of Burkitt's lymphoma (which is a cancer of the lymphatic system) cells in combination with Gemcitabine (Figure 1) treatment.

Materials and methods

Cell culture and 1.8 GHz MW radiation exposure

Human Burkitt's lymphoma Raji cell line was obtained from the Foot-and-Mouth Disease Institute of Ministry of Agriculture & Rural Affairs of Turkey. The cells were maintained in suspension cultures in RPMI 1640 medium

Address correspondence to Dr Meric Arda Esmekaya, Department of Biophysics, Faculty of Medicine, Gazi University, Ankara, Turkey. E-mail: mericarda@yahoo.com

(Hyclone, Thermo, Waltham, MA) containing 2 mM L-glutamine, 100 U/mL penicillin G, 100 mg/mL streptomycin and 10% heat inactivated fetal bovine serum (FBS) (all obtained from Hyclone, Thermo, Waltham, MA) in a 37 °C humidified atmosphere with 5% CO_2.

A 1.8 GHz MW signal was produced by a vector signal generator. The signal generator was connected to a horn antenna. The antenna which radiated upwards was settled in a CO_2 incubator vertically and cells were placed above this antenna (Figure 2). The temperature inside the incubator was kept constant at 37 °C. The electric field strength which was measured by a handheld spectrum analyzer was 40 V/m. The duration of exposure was 24 h. Sham-control samples were in the same conditions as MW exposed samples but generator was off.

In order to characterize the response of the cell suspension to the MW exposure, numerical analysis was performed. The SAR distribution inside the eppendorf tubes was calculated by the SEMCAD X (Schmid & Partner Engineering AG, Zurih, Switzerland) commercial software, solving Maxwell's equations based on the Finite-Difference Time-Domain (FDTD) method. The numerically modeled geometry is composed of the horn antenna, eppendorf tubes containing homogeneous biological suspension, and the holder of the eppendorf tubes (Figure 3). Dielectric properties of the materials used in simulations are given in Table 1. Eppendorf tubes were filled with 1 ml each of the culture medium. Numerical models for medium inside eppendorf tubes were used to assess 10 g averaged SAR values centered on 1 mm above the horn antenna at 1.8 GHz. Grading mesh algorithm was used to reduce the number of voxels and computational time in simulation. The minimum and maximum grid steps were 1.332e-04 and 8.108e-03, respectively. The simulation consisted of 40.67 million voxels. The Perfectly Matched Layer (PML) absorbing boundary condition was used to truncate FDTD lattices. SAR value was obtained by normalizing antenna input power to 1 Watt. 10 g peak spatial average SARs ranged between 0.336 and 0.375 W/kg at 1.8 GHz Table 2.

Cell viability

Cell viability was quantified using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Cells were diluted with culture medium to the seeding density, suspended in 96-well tissue culture plates (200 µl, containing 10^4 cells, per well), and preincubated at 37 °C overnight. Raji cells were incubated with 5 µM gemcitabine 24 h before MW radiation exposure. When MW exposures ended, 10 µl of

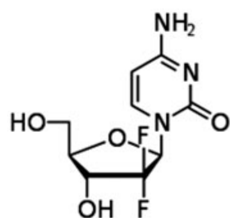

Figure 1. Molecular structure of Gemcitabine.

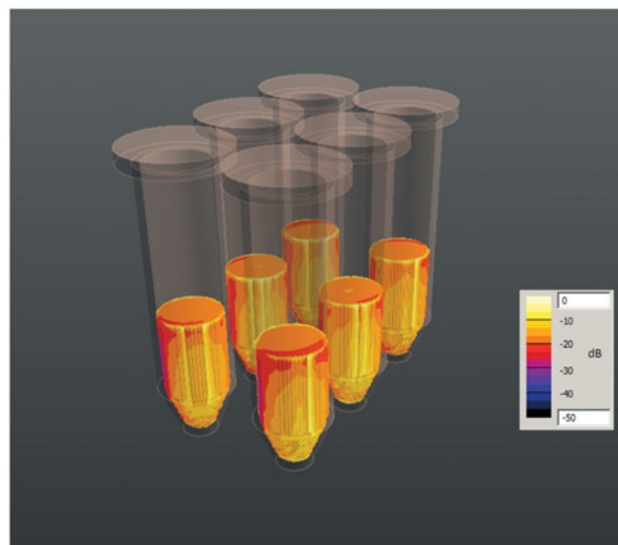

Figure 3. Simulated SAR distribution for cell suspension in the eppendorf tubes, exposure at 1.8 GHz.

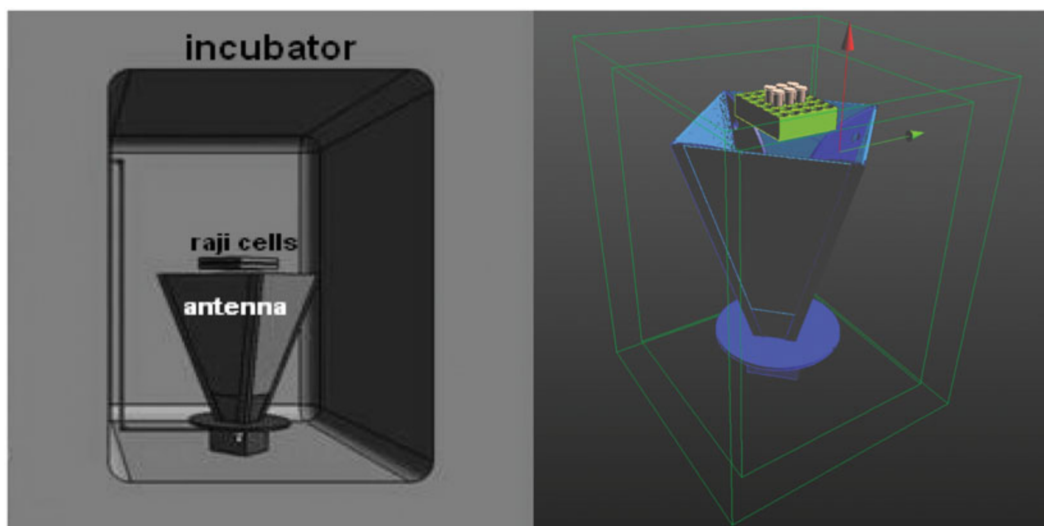

Figure 2. The MW exposure system.

Electromagn Biol Med Downloaded from informahealthcare.com by Gazi Univ. on 06/09/14
For personal use only.

Table 1. Dielectric properties of materials used in the simulation at 1.8 GHz.

Component of modeling	ε_r	σ (S/m)	d (kg/m^3)
Pleksiglass surface of horn antenna	2.4	0	–
Polystyrene box and eppendorf tubes	2.6	0	–
Cell culture	75	2.2	1060

Table 2. Simulated SAR Values for cell suspension in the eppendorf tubes, exposure at 1.8 GHz.

Materials	10 g SAR (W/kg)
1. Eppendorf Tube	0.336
2. Eppendorf Tube	0.341
3. Eppendorf Tube	0.370
4. Eppendorf Tube	0.375
5. Eppendorf Tube	0.339
6. Eppendorf Tube	0.336
Average	0.350

MTT (5 mg/ml) was added to each well. After 4 h of incubation with MTT, 100 µl of DMSO was added and the absorbance of the MTT-formazan product at 570 nm was measured with a Spectramax M3 microplate reader (Molecular Devices, Sunnyvale, CA) after dissolving the crystals in DMSO. All experiments were repeated five times for each dose and the mean absorbance values were calculated.

Apoptosis detection by flow cyrometry analyses

The amount of apoptotic cells was analyzed by Annexin V-FITC and propidium iodide (PI) staining. Following to MW exposure, cells were washed with PBS by gentle shaking and pipetting up and down and then resuspended in binding buffer. 5 µl Annexin-V-FITC was added to 195 µl cell suspension. The cells were then vortexed and incubated for 10 min at room temperature. Atferwards, cells were washed again and resuspended in 190 µl binding buffer and 10 µl PI (20 µg/ml) was added into solution. Samples were analyzed by a FacsCalibur Flow Cytometer (Becton-Dickinson, San Jose, CA) using the Cell Quest software (Becton-Dickinson, San Jose, CA). Annexin–V positive cells were considered as apoptotic (Han et al., 2008; Sumit and Paul, 2005.

Results

Apoptosis and cell viability results

The apoptotic activity of MW-exposed Raji cells was increased significantly when compared with sham exposed samples ($p < 0.05$), Moreover, cell viability of exposed samples (Figure 4) was decreased ($p < 0.05$). Combined exposure of MW radiation and Gemcitabine treatment further significantly increased the amount of apoptotic cells (Figure 5) than MW radiation alone ($p < 0.05$). Moreover, viability of MW + Gemcitabine exposed cells was lower than MW-exposed cells alone ($p < 0.05$).

Discussion

Gemcitabine is used alone or in combination with other chemotherapeutic drugs to treat various types of cancer,

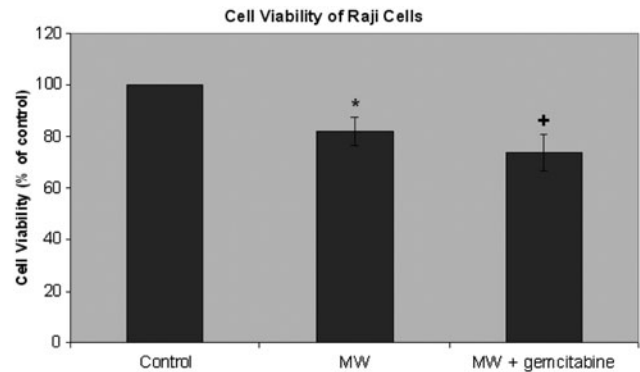

Figure 4. Relative cell viability (%) of Raji cells (viability of control = 1). The percent viability was determined by the MTT reduction assay. The data are presented as the mean ± SD. *Significantly different from control, +significantly different from control and MW.

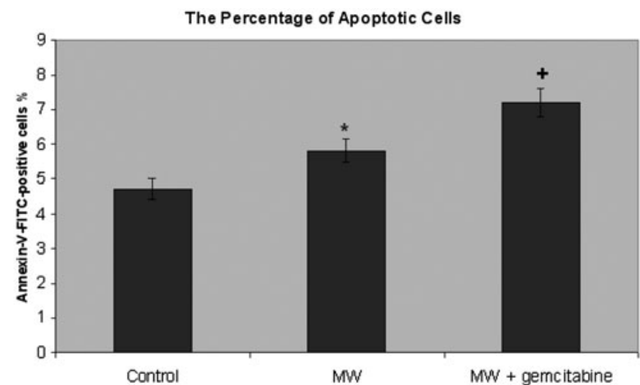

Figure 5. Percentage of Annexin-V positive Raji cells in control and exposed groups. Cells were analyzed by flow cytometry with Annexin-V and PI to determine the percentage of apoptosis. Vertical bars indicate percent of cells SD in each group. *Significantly different from control, +significantly different from control and MW.

including lung, pancreatic, bladder and breast cancers and experimentally used in lymphomas and other cancer types. It is metabolized intracellularly to two active metabolites, gemcitabine diphosphate (dFdCDP) and gemcitabine triphosphate (dFdCTP). The cytotoxic effects of gemcitabine are exerted through incorporation of dFdCTP into DNA with the assistance of dFdCDP. It exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis in S-phase and under certain conditions blocking progression of cells through the GI/S-phase boundary. It inhibits thymidylate synthetase and causes inhibition of DNA synthesis and apoptosis

In the present study, we investigated whether MW radiation with/without Gemcitabine treatment activated apoptotic pathways in Raji cells. Our results showed that 1.8 GHz GSM-modulated MW radiation for 24 h caused apoptotic cell death. In addition, combined exposure of MW radiation and Gemcitabine treatment increased the amount of apoptotic cells than MW radiation alone. The viability of Raji cells was significantly decreased due to MW exposure. Moreover, viability of MW + Gemcitabine exposed cells was lower than MW exposed cells alone.

Electromagn Biol Med Downloaded from informahealthcare.com by Gazi Univ. on 06/09/14
For personal use only.

Electromagn Biol Med Downloaded from informahealthcare.com by Gazi Univ. on 06/09/14
For personal use only.

Jin et al. (2012) researched the effects of 900 MHz MW radiation in combination with a chemotherapeutic drug, doxorubicin (DOX) at a dose of 0.125 mg/L in leukemia HL-60 cells. Cell cultures were exposed to 12 $\mu W/cm^2$ MW radiation power density for 1 h/day for 3 days with and without DOX treatment and measured several parameters including cell viability, apoptosis and mitochondrial membrane potential ($\Delta\Psi m$). Different from the results of our study, no significant change was found in terms of cell viability and apoptosis between unexposed and MW exposed samples. However, DOX treatment caused a significant decrease in cell viability and increased rate of apoptosis. Moreover, they observed increased cell viability and $\Delta\Psi m$ and decreased apoptosis in MW + DOX exposed cells. MW radiation exposure increased apoptotic rate and decreased proliferative capacity of thymocytes in rats (Sokolovic et al., 2013). A 15-min exposure to 5 and 10 mW/cm^2 continuous MW radiation at a frequency of 2.45 GHz increased apoptosis and the proportion of aberrant spindles in V79 Chinese hamster cells. On the contrary, mitotic index was decreased due to MW radiation (Ballardin et al., 2011). The study of Joubert et al. (2008) reported a significant increase in the percentage of apoptotic cells in neurons when the cells were exposed to a 900 MHz CW MW radiation (2 W/kg) for 24 h. The study also showed that apoptosis-inducing factor was increased significantly in MW exposed cells.

The expression of apoptotic genes is also affected by MW radiation exposure in different cell types and exposure conditions. Karaca et al. (2012) investigated whether gene expression of some proapoptotic and antiapoptotic genes were affected by MW radiation. They exposed brain cell cultures of the mice to 10.715 GHz at a SAR level of 0.725 W/kG signals for 6 h in 3 days. STAT3 expression decreased 7-fold due to MW radiation. Moreover, MN frequency inreased 11-fold in MW-exposed cells. 2.45 GHz MW radiation increased c-Fos protein expression in the paraventricular nucleus of rat hypothalamus (Mora et al., 2011). Buttiglione et al. (2007) used a wire patch cell (WPC) antenna that transmits MW fields to investigate if MW radiation may induce apoptosis and alter the expression of some apoptotic proteins in SH-SY5Y neuroblastoma cells. 900 MW radiation that was generated by a WPC antenna resulted in a significant decrease in mRNA levels of Bcl-2 and survivin genes. In addition, cell proliferation was inhibited and cell cycle progression was impaired. MW radiation induced a G2-M arrest. The study of Pacini reported that an one-hour exposure to GSM radiation may increase the expression of mitogenic signal transduction genes in human skin fibroblasts (Pacini et al., 2002).

The present study reported that GSM-modulated MW radiation at frequency of 1.8 GHz induced apoptosis. Moreover, Gemcitabine treatment increased apoptotic activity of MW exposed cells. MW radiation exposure and Gemcitabine treatment have a synergistic effect on Raji cells.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

Ballardin, M., Tusa, I., Fontana, N., et al. (2011). Non-thermal effects of 2.45 GHz microwaves on spindle assembly, mitotic cells and viability of Chinese hamster V-79 cells. *Mutat. Res.* 716:1–9.

Buttiglione, M., Roca, L., Montemurno, E., et al. (2007). Radiofrequency radiation (900 MHz) induces Egr-1 gene expression and affects cell-cycle control in human neuroblastoma cells. *J. Cell Physiol.* 213: 759–767.

Esmekaya, M. A., Aytekin, E., Ozgur, E., et al. (2011). Mutagenic and morphologic impacts of 1.8 GHz radiofrequency radiation on human peripheral blood lymphocytes (hPBLs) and possible protective role of pre-treatment with Ginkgo Biloba (EGb 761). *STOTEN.* 410: 59–64.

Fesenko, E. E., Makar, V. R., Novoselova, E. G., Sadovinikov, V. B. (1999). Microwaves and cellular immunity. I. Effect of whole body microwave irradiation on tumor necrosis factor production in mouse cells. *Bioelectrochem. Bioenerg.* 49:29–35.

Han, Y. H., Kim, S. H., Kim, S. Z., Park, W. H. (2008). Antimycin A as a mitochondrial electron transport inhibitor prevents the growth of human lung cancer A549 cells. *Oncol. Rep.* 20:689–693.

Hardell, L., Carlberg, M., Mild, K. H. (2006a). Case-control study of the association between the use of cellular and cordless telephones and malignant brain tumors diagnosed during 2000–2003. *Environ. Res.* 100:232–241.

Hardell, L., Mild, K. H., Carlberg, M., et al. (2006b). Tumour risk associated with use of cellular telephones or cordless desktop telephones. *World J. Surg. Oncol.* 4:74. doi: 10.1186/1477-7819-4-74.

Jin, Z., Zong, C., Jiang, B., et al. (2012). The effect of combined exposure of 900 MHz radiofrequency fields and doxorubicin in HL-60 cells. *PLoS One.* 7:e46102.

Mora, J. T., Misa-Agustiño, M. J., Rodríguez-González, J. A., et al. (2011). The effects of single and repeated exposure to 2.45 GHz radiofrequency fields on c-Fos protein expression in the paraventricular nucleus of rat hypothalamus. *Neurochem. Res.* 36:2322–2332.

Joubert, V., Bourthoumieu, S., Leveque, P., Yardin, C. (2008). Apoptosis is induced by radiofrequency fields through the caspase-independent mitochondrial pathway in cortical neurons. *Radiat. Res.* 169:38–45.

Karaca, E., Durmaz, B., Aktug, H., et al. (2012). The genotoxic effect of radiofrequency waves on mouse brain. *J. Neurooncol.* 106:53–58.

Lai, H., Singh, N. P. (1995). Acute low intensity microwave exposure increases DNA single-strand breaks in rat brain cells. *Bioelectromagnetics.* 16:207–210.

Litovitz, T. A., Krause, D., Mullins, J. M. (1993). The role of coherence time in the effect of microwaves on ornithine decarboxylase activity. *Bioelectromagnetics.* 14:395–403.

Litovitz, T. A., Penafiel, L. M., Farrel, J. M., et al. (1997a). Bioeffects induced by exposure to microwaves are mitigated by superposition of ELF noise. *Bioelectromagnetics.* 18:422–430.

Litovitz, T. A., Penafiel, M., Krause, D., et al. (1997b). The role of temporal sensing in bioelectromagnetic effects. *Bioelectromagnetics.* 18:388–395.

Maes, A., Verschaeve, L., Arroyo, A., et al. (1993). In vitro cytogenetic effects of 2450 MHz waves on human peripheral blood lymphocytes. *Bioelectromagnetics.* 14:495–501.

Nemoto, T., Hori, H., Yoshimoto, M., et al. (2002). Overexpression of ornithine decarboxylase enhances endothelial proliferation by suppressing endostatin expression. *Blood.* 15:1478–1481.

Pacini, S., Ruggiero, M., Sardi, I., et al. (2002). Exposure to global system for mobile communication (GSM) cellular phone radiofrequency alters gene expression, proliferation, and morphology of human skin fibroblasts. *Oncol. Res.* 13:19–24.

Penafiel, M. L., Litovitz, T. A., Krause, D., et al. (1997). Role of modulation on the effect of microwaves on ornithine decarboxylase activity in L929 cells. *Bioelectromagnetics.* 18:132–141.

Repacholi, M. H., Basten, A., Gebski, V., et al. (1997). Lymphomas in E mu-Pim1 transgenic mice exposed to pulsed 900 MHZ electromagnetic fields. *Radiat. Res.* 147:631–640.

Sokolovic, D., Djordjevic, B., Kocic, G., et al. (2013). Melatonin protects rat thymus against oxidative stress caused by exposure to microwaves and modulates proliferation/apoptosis of thymocytes. *Gen. Physiol. Biophys.* 32:79–90.

Sumit, J. S., Paul, W. S. (2005). Tocotrienol-induced cytotoxicity is unrelated to mitochondrial stress apoptotic signaling in neoplastic mammary epithelial cells. *Biochem. Cell Biol.* 83:86–95.

Veyret, B., Bouthet, C., Deschaus, P., et al. (1991). Antibody responses of mice exposed to low-power microwaves under combined pulse-and-amplitude modulation. *Bioelectromagnetics.* 12:47–56.

Yakymenko, I., Sidorik, E., Kyrylenko, S., Chekhun, V. (2011). Long-term exposure to microwave radiation provokes cancer growth: Evidences from radars and mobile communication systems. *Exp. Oncol.* 33:62–70.

Electromagn Biol Med Downloaded from informahealthcare.com by Gazi Univ. on 06/09/14
For personal use only.