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THE EFFECT OF MICROWAVE EMISSION FROM MOBILE PHONES ON NEURON SURVIVAL IN RAT CENTRAL NERVOUS SYSTEM

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Abstract—To investigate the effect of microwave emitted by mobile phones on the rat central nervous system (CNS), *in vitro* cultured cortical neuronal cells and *in vivo* rat's brain were exposed to the electromagnetic waves emitted by a microwave transmitter that mimics the working frequency of mobile phones. Trypan blue staining and terminal deoxynucleotidy transferase-mediated dUTP nick-end labeling (TUNEL) were used to determine the survival state of neuronal cells while immunohistochemistry method was used to determine the expression level of Bcl-2 and Bax. Our results show that microwave lead to significant cell death in culture and more *in vivo* brain neuronal cells were stained positive for TUNEL, Bax and Bcl-2 in rats with cranial defect after exposure than that for

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control groups (with intact cranium, or had no microwave exposure) ($P < 0.01$). However, no significant differences were observed in the ratio of Bax/Bcl-2 among the groups studied. Therefore, microwave emitted from mobile phones is harmful to both *in vitro* cultured cortical cells and *in vivo* brain neuronal cells from rat with cranial defect. The integrity of cranium is important in protecting the CNS against apoptotic injuries inflicted by the microwaves from mobile phones.

1. INTRODUCTION

In the recent years, the widespread use of mobile phones has given rise to a growing concern over their possible adverse effects of the electromagnetic waves emitted on human health [1–6]. In addition to those obvious life-threatening hazards of using a mobile phone under certain conditions, such as mobile phone use while operating an automobile or the unwanted interferences with medical equipment or implanted pacemakers by mobile phone radiation, the low intensity, pulsed microwave radiation that the GSM mobile phones emit can also exert subtle, non-thermal influences on the organism. Since mobile phones are usually held close to the head when they are in use, part of the microwave they emit is absorbed by the brain [7–9]. For example, most microwaves in the frequency range of 800–1000 MHz can penetrate the cranium and near 40% of them can reach the deep brain [1, 4, 5, 10–13]. Therefore, it is conceivable that microwave from mobile phones could affect brain functions. As a matter of fact, preliminary studies have already reported that mobile phones affect brain functioning and behaviors [14–25]. For example, the electromagnetic field generated by a GSM mobile phone modifies brain excitability and enhances electroencephalogram alpha-band power. However, current studies mainly address the potential adverse biological effects of mobile phone microwave, while lacks demonstration of the basic mechanisms behind these biological effects. Therefore, in this study, we used a microwave device to mimic the microwave emitted by mobile phones and investigated its bio-effects on the survival of both *in vitro* cultured rat neuronal cells and *in vivo* rat CNS neuronal cells. However, we do realize the human body, as an integral organism, has much stronger resistance and powerful defense against outside insults like microwave than the *in vitro* cultured cells. Accordingly, we then designed an *in vivo* rat model to study the effect of microwave emitted from mobile phones on the *in vivo* CNS neuronal cells and explore the role cranium may play during that process.

2. MATERIALS AND METHODS

2.1. Microwave Generator System

The experimental setup is shown in Fig. 1, where the electromagnetic waves are radiated by a horn antenna. The signal is generated by a Microwave generator (8674A, Hewlett Packard), the electromagnetic signal is firstly amplified by a Power Amplifier (Model 727LC-CE, Kalmus) before it is fed to the horn antenna. The field densities in the chamber are measured by the microwave analyzer (ESA-1500A, Hewlett Packard). The radiation frequency is set to be 900 MHz.

2.2. Exposure of Neuronal Culture to Microwave

2.2.1. Primary Cortical Neuronal Culture

All animal research protocols were approved by the Animal Care and Use Committee of Zhejiang University School of Medicine. Rat cortical neuronal cells were prepared from the cerebral cortex of neonatal (P 0-1) Sprague-Dawley (SD) rats at the Experimental Animal Center of Zhejiang University School of Medicine as previously described [26]. Briefly, rats were decapitated under deep anaesthesia, and their brains were quickly removed and placed in ice-cold dissociation solution, the Ca^{2+}, Mg^{2+} and bicarbonate-free Hanks' balanced salt solution (CMF-Hanks'; Gibco, Paisley, UK). Cerebral cortex was isolated using fine tweezers and chopped into small pieces that were then placed in a treatment chamber containing 0.25% trypsin in dissociation solution at 37°C. After 30 min incubation, the tissue was rinsed several times in dissociation solution and mechanically dissociated using a series of fire-polished Pasteur pipettes. After centrifuge, the supernatant was discarded and the dissociated neuronal cells were collected and seeded into tissue culture plate pre-coated with polylysine at a density of 10^6 cells/ml. Cells were allowed to settle for 1 hour at 37°C before the dissociation solution was replaced with culture medium (DMED supplemented with 15% deactivated bovine serum, Invitrogen, USA) and cultures were maintained at 37°C, 5% CO_2. 48 hours later, 10 µM cytarabine was added to inhibit the growth of non-neuronal cells. Culture medium was replaced with fresh ones twice a week until the cells were exposed to microwave 12 days later.

2.2.2. Microwave Exposure

Three different power densities were selected (0.025 mW/cm^2, 0.05 mW/cm^2 and 0.1 mW/cm^2) and culture plate in the incubator were directly exposed to the microwaves through a microwave probe for

different duration (4 h, 8 h, 12 h, 16 h, 20 h and 24 h). After microwave exposure, cells were stained with a Typan Blue Staining Cell Viability Assay Kit and observed under an inverted phase contrast microscope to distinguish dead cells from live cells. The percentage of dead cell (stained cell) was calculated.

2.3. *In Vivo* Exposure of Rat Brain to Microwave

2.3.1. Animal Model Establishment

80 male SD rats, weighing 200–250 g, were purchased from Experimental Animal Center of Zhejiang University School of Medicine and feed in our laboratory for 1 w to adapt to new environment. Rats were randomly divided into four groups: blank group (without cranial defect or microwave exposure), pure radiation group (with intact cranium), pure operation group (with cranial defect but no microwave exposure) and radiation plus operation group (with cranial defect and microwave exposure). The cranial defect is made to the left of the center point between anterior fontanel and lambda with a diameter of 5.0 mm. To minimize the influences by surgery or anesthesia itself, rats were left free in their cages for 72 h to recover their normal activities such as feeding and walking before microwave exposure. The exposure method is adapted from previous study [27] with some modifications (Fig. 1). Briefly, the rats were loaded into a polymethyl methacrylate plastics cage and received systematical

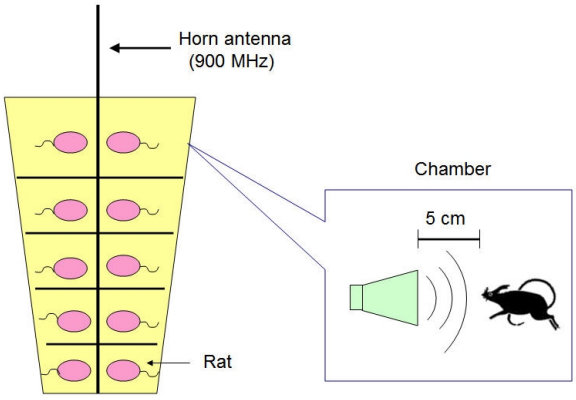

Figure 1. Scheme of radiation mimicking mobile phone by horn antenna to rats in plastic chamber. Rats were loaded into plastics cage, confined by the chamber's wall, and received systematical microwave exposure rostrocaudally as illustrated in the figure.

microwave exposure rostrocaudally. Their movements were confined by the wall of the cage and their heads were 5 cm away from the irradiation bomb. Microwave exposure was given twice a day (2 h in the morning and 2 h in the afternoon), for 21 consecutive days. The control animals were treated similarly except for the exposure to microwave.

2.3.2. Detection of Apoptosis

Rats were sacrificed under deep anesthesia and their brains were collected and fixed in 4% paraformaldehyde for 6 h. Paraffin-embedded rat brain sections were prepared according to routine procedures. Slides were stained with terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay and Streptavidin-peroxidase (SP) immunohistochemistry assay to determine the apoptotic state of brain neuronal cells as well as their expression level of Bcl-2 and Bax. Cells showed buffy or brownish red granules in their cytoplasm were regarded as positive. Labeling Index (LI) was calculated according to the following formula: LI = (positive cell number/total cell count) $\times$ 100%.

2.4. Statistics

Data were presented as mean $\pm$SD and statistical analyses were evaluated by Two-Way ANOVA (SPSS 10.0 software). P value < 0.05 is considered as statistically significant.

3. RESULTS

After exposure to microwave, the cultured cortical neuronal cells were allowed to continue growth for 48 h before typan blue staining. We found no significant difference between 0.025 mW/cm^2 power density group and control group in the percentage of dead cell after microwave exposure. However, the percentage of dead cell reached 11.70 ± 2.67% when the cells were exposed at 0.05 mW/cm^2 power density for 12 h, which is much higher than that of the control group. Similarly, exposure at 0.1 mW/cm^2 power density for 8 h also leads to significantly higher cell death rate (14.62 ± 2.43%) than that of the control group. Moreover, in both 0.05 mW/cm^2 and 0.1 mW/cm^2 power density groups, more cell died as the exposure time prolonged (Fig. 2).

According to TUNEL and SP immunohistochemistry assay, few cells were positive for TUNEL, Bcl-2 and Bax in the blank group. However, positive cell number slightly increased in pure radiation

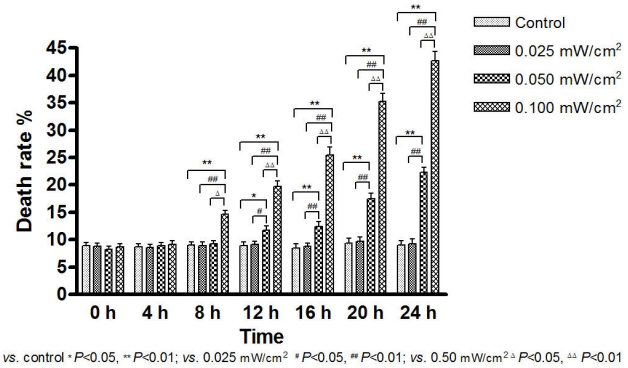

Figure 2. The effects of microwave emitted from mobile phones on the survival of *in vitro* cultured rat cortical neuronal cells ($n = 12$). Microwave exposure at $0.05\,\mathrm{mW/cm^2}$ power density for 12 h, or $0.1\,\mathrm{mW/cm^2}$ power density for 8 h led to significant cell death, and more cell died as the exposure time prolonged. The mortality of neuronal cells was determined by typan blue staining.

group and pure operation group and was highest in radiation plus operation group. There was no significant difference in the percentage of positive cell for TUNEL, Bcl-2 and Bax among blank group, pure radiation group and pure operation group ($P > 0.05$). However, the differences between radiation plus operation group and other three control groups were significant ($P < 0.01$). Because Bcl-2 and its homolog Bax show opposite role in the regulation of apoptosis (the former is an anti-apoptotic protein that protects cells from apoptosis whereas the latter functions as a pro-apoptotic protein which kills cells) and as we have noticed both proteins were up-regulated during the early phase after microwave exposure in the radiation plus operation group, therefore we measured the expression level of Bcl-2 and Bax in all groups. However, there is no significant difference in the ratio of Bax/Bcl-2 among the groups after microwave exposure ($P > 0.05$) (**Fig. 3**).

4. DISCUSSION

Microwaves from mobile phones induced neuronal death both *in vivo* and *in vitro*

In this present work, we used the neonatal rat cortical neuronal culture as an *in vitro* model to explore the potential CNS injury

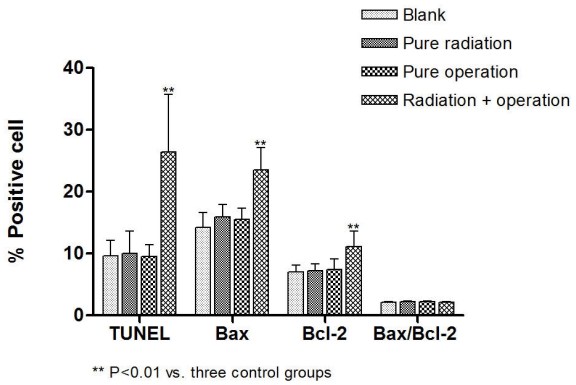

Figure 3. TUNEL and immunohistochemistry determination of Bcl-2 and Bax level in the rats exposed to microwave. After microwave exposure, the percentage of positive cell for TUNEL, Bcl-2 and Bax were significantly higher in rats received radiation plus operation than the three control groups ($P < 0.01$). However, there is no significant difference in the ratio of Bax/Bcl-2 among the groups after microwave exposure ($P > 0.05$).

inflicted by microwave exposure. Our results showed that exposed at 0.05 mW/cm^2 power density for 12 h or 0.1 mW/cm^2 power density for 8 h led to significant neuronal deaths and the extend of cell death correlates with the length of exposure. These results indicate that chronic exposure to a low-intensity microwave radiation has lethal effect to the rat neuronal cells.

In vivo neuronal cell death in the form of apoptosis after microwave exposure

Apoptosis is characterized with DNA fragmentation. TUNEL method [28] was developed for detecting *in situ* apoptosis at the single-cell level, while preserving tissue architecture. Conventional histological sections, pretreated with protease, were nick end labeled with biotinylated poly dU, introduced by terminal deoxy-transferase, and then stained using avidin-conjugated peroxidase. The reaction is specific, only nuclei located at positions where apoptosis is expected are stained. Our experiments using TUNEL assay showed microwave exposure, though induced significant neuronal apoptosis in the radiation plus operation group (rats with cranial defect), led to little neuronal cell apoptosis in the pure radiation group (rat with intact cranium). Because cell apoptosis is an early marker for cell injury and necrosis, our results indicate intact cranium seems to be

important in protecting the CNS against microwave injury.

Factors besides Bax and Bcl-2 were involved in the regulation of apoptosis

The bcl-2 gene belongs to a group of proto-oncogenes that promote cell survival by counteracting the process of apoptosis [29]. The bax gene is an apoptosis-promoting member of the bcl-2 gene family [30]. Bcl-2 protein has the unusual property of increasing cell numbers by preventing apoptosis and the death-protective activity of Bcl-2 seems to be proportional to its expression level, whereas Bax protein induces apoptosis when over-expressed in a variety of eukaroytic cells. Bax protein forms homodimers and heterodimers with Bcl-2 *in vivo* and can induce Bcl-2-inhibited apoptosis. It is thought that the molar ratio of Bcl-2 to Bax determines whether apoptosis is induced or inhibited in several tissues [31–33]. Our results showed Bax protein was detectable even in the blank group and was significantly up-regulated early after microwave exposure in the radiation plus operation group when compared with the control groups (blank group, pure radiation group and pure operation group). These results indicate Bax is involved in the cellular apoptosis induced by microwave exposure. Interestingly, Bcl-2 also showed similar expression pattern (detectable in the blank group while highest in the radiation plus operation group). Therefore, anti-apoptotic protein Bcl-2 also participates in the cellular apoptosis induced by microwave exposure. As mentioned above, Bcl-2 act as death repressors whereas Bax exert the opposite function. And Bcl-2 protein forms heterodimers with the Bax protein *in vivo*, and the Bcl-2-Bax heterodimer disrupts the anti-apoptosis function of Bcl-2. Therefore, the percentage of Bcl-2 to Bax determines whether apoptosis is induced or inhibited in many tissues. However, we found Bax/Bcl-2 ratio is relatively stable in all groups studied at all time points, indicating the balance between Bax pro-apoptotic effect and Bcl-2 anti-apoptotic effect is largely preserved. Therefore, Bax/Bcl-2 ratio seems not to be the most determinant factor on whether or not neuronal cell apoptosis is induced after microwave exposure and other factors might also be involved in the regulation of these apoptosis processes.

Intact cranium is an important defensive factor against apoptotic injury by microwave

Because removing part of the cranium itself is a traumatic insult on the rat brain and, in order to minimize the influences of surgery on our results, the microwave exposure is delayed to 72 h after operation and a pure operation group (without microwave exposure) is established as control. Our results indicated that the surgery has

limited impact on the cellular apoptosis in the rat CNS since no significant difference was observed between pure operation group and blank group in terms of percentage of apoptosis.

According to our results, the intact cranium is an important protective factor in fortifying the *in vivo* CNS defenses against microwave injury by mobile phones since the cranium, partly because it can reflect and absorb some of the microwave, whereas the existence of cranial defect may render the CNS to be more susceptible to microwave injury. On the basis of these findings, we conclude, thought the daily usage of mobile phones might not induce significant adverse microwave injury in healthy individual, we should take cautions when extending this conclusion to patients with cranial defect. At present, more and more people suffered from cranial defects as a result of traffic accidents or CNS tumors and the defective cranium might further render these patients' CNS more susceptible to outside insulting factors, e.g., microwave emitted from mobile phones.

5. CONCLUSIONS

Although the specific mechanism mediating the microwave induced neuronal apoptosis is still uncertain, our present study not only showed that the *in vitro* cultured cortical neuronal cells and *in vivo* CNS with cranial defect were susceptible to apoptotic injury by mobile phone microwave, but also indicated that factors beside Bax and Bcl-2 were involved in the regulation of this apoptosis process. We believe future efforts made to explore the basic mechanism underling the mobile phone microwave induced CNS injuries would be especially important for patients who have cranial defects as a result of traumatic brain injury or brain tumor.

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REFERENCES

1. Barnett, J., L. Timotijevic, R. Shepherd, and V. Senior, "Public responses to precautionary information from the Department of Health (UK) about possible health risks from mobile phones," *Health Policy*, Vol. 82, No. 2, 240–250, 2007.
2. Karger, C. P., "Mobile phones and health: A literature overview," *Z. Med. Phys.* Vol. 15, No. 2, 73–85, 2005.

3. Wu, B. I., F. C. A. I. Cox, and J. A. Kong, "Experimental methodology for non-thermal effects of electromagnetic radiation on biologics," *Journal of Electromagnetic Waves and Applications*, Vol. 21 No. 4, 533–548, 2007.
4. Stilgoe, J., "The (co-) production of public uncertainty: UK scientific advice on mobile phone health risks," *Public Underst. Sci.*, Vol. 16, No. 1, 45–61, 2007.
5. Elder, A. J., C.-K. Chou, and J. J. Morrissey, "Radiofrequency exposure and human health," *PIERS Online*, Vol. 3, No. 2, 149–153, 2007.
6. Sakari, L., "Recent advances in bioelectromagnetics research on mobile telephony and health — An introduction," *PIERS Online*, Vol. 2, No. 2, 192–196, 2006.
7. Kouveliotis, N. K., S. C. Panagiotou, P. K. Varlamos, and C. Capsalis, "Theoretical approach of the interaction between a human head model and a mobile handset helical antenna using numerical methods," *Progress In Electromagnetics Research*, PIER 65, 309–327, 2006.
8. Ismail, N. H. and A. T. Ibrahim, "Temperature distribution in the human brain during ultrasound hyperthermia," *Journal of Electromagnetic Waves and Applications*, Vol. 16, No. 6, 803–811, 2002.
9. Lang, S., "Recent advances in bioelectromagnetics research on mobile telephony and health — An introduction," *PIERS Online*, Vol. 2, No. 2, 192–196, 2006.
10. Kuo, L. C., Y. C. Kan, and H. R. Chuang, "Analysis of a 900/1800-MHz dual-band gap loop antenna on a handset with proximate head and hand model," *Journal of Electromagnetic Waves and Applications*, Vol. 21, No. 1, 107–122, 2007.
11. Klemm, M. and G. Troester, "EM energy absorption in the human body tissues due to UWB antennas," *Progress In Electromagnetics Research*, PIER 62, 261–280, 2006.
12. Kang, X. K., L. W. Li, M. S. Leong, and P. S. Kooi, "A method of moments study of SAR inside spheroidal human head and current distribution along handset wire antennas," *Journal of Electromagnetic Waves and Applications*, Vol. 15, No. 1, 61–75, 2001.
13. Khalatbari, S., D. Sardari, A. A. Mirzaee, and H. A. Sadafi, "Calculating SAR in two models of the human head exposed to mobile phones radiations at 900 and 1800 MHz," *PIERS Online*, Vol. 2, No. 1, 104–109, 2006.

14. Ferreri, F., G. Curcio, P. Pasqualetti, L. de Gennaro, R. Fini, and P. M. Rossini, "Mobile phone emissions and human brain excitability," *Ann. Neurol.* Vol. 60, No. 2, 188–196, 2006.
15. Hamblin, D. L. and A. W. Wood, "Effects of mobile phone emissions on human brain activity and sleep variables," *Int. J. Radiat. Biol.*, Vol. 78, No. 8, 659–669, 2002.
16. Khiat, A., Y. Boulanger, and G. Breton, "Monitoring the effects of mobile phone use on the brain by proton magnetic resonance spectroscopy," *Int. J. Radiat. Biol.*, Vol. 82, No. 9, 681–685, 2006.
17. Lonappan, A., V. Thomas, G. Bindu, C. Rajasekaran, and K. T. Mathew, "Analysis of human cerebro spinal fluid at the ISM band of frequencies," *Journal of Electromagnetic Waves and Applications*, Vol. 20, No. 6, 773–779, 2006.
18. Krause, C. M., M. Pesonen, C. Haarala-Bjornberg, and H. Hamalainen, "Effects of pulsed and continuous wave 902 MHz mobile phone exposure on brain oscillatory activity during cognitive processing," *Bioelectromagnetics*, Vol. 28, No. 4, 296–308, 2007.
19. Kumlin, T., H. Iivonen, P. Miettinen, A. Juvonen, T. van Groen, L. Puranen, R. Pitkaaho, J. Juutilainen, and H. Tanila, "Mobile phone radiation and the developing brain: Behavioral and morphological effects in juvenile rats," *Radiat Res.*, Vol. 168, No. 4, 471–479, 2007.
20. Lonn, S., A. Ahlbom, P. Hall, and M. Feychting, "Long-term mobile phone use and brain tumor risk," *Am. J. Epidemiol.*, Vol. 161, No. 6, 526–535, 2005.
21. Sievert, U., S. Eggert, and H. W. Pau, "Can mobile phone emissions affect auditory functions of cochlea or brain stem?" *Otolaryngol. Head Neck Surg.*, Vol. 132, No. 3, 451–455, 2005.
22. Ebrahimi-Ganjeh, M. A. and A. R. Attari, "Interaction of dual band helical and PIFA handset antennas with human head and hand," *Progress In Electromagnetics Research*, PIER 77, 225–242, 2007.
23. Johnston, A. S., "Behavioral and cognitive effects of MW electromagnetic field exposures," *PIERS Online*, Vol. 3, No. 5, 751–758, 2007.
24. Persson, R. R. B., J. Eberhardt, L. Malmgren, B. M. Persson, A. Brun, and G. L. Salford, "Effects of microwaves from GSM mobile phones on the blood-brain barrier and neurons in rat brain," *PIERS Online*, Vol. 1, No. 6, 638–641, 2005.
25. Sadafi, A. H., Z. Mehboodi, and D. Sardari, "A review of the

mechanisms of interaction between the extremely low frequency electromagnetic fields and human biology," *PIERS Online*, Vol. 2, No. 1, 99–103, 2006.

26. Lin, P. W., C. C. Wu, C. H. Chen, H. O. Ho, Y. C. Chen, and M. T. Sheu, "Characterization of cortical neuron outgrowth in two- and three-dimensional culture systems," *J. Biomed. Mater. Res. B. Appl. Biomater.*, Vol. 75, No. 1, 146–157, 2005.
27. Marino, C., G. Cristalli, P. Galloni, P. Pasqualetti, M. Piscitelli, and G. A. Lovisolo, "Effects of microwaves (900 MHz) on the cochlear receptor: Exposure systems and preliminary results," *Radiat. Environ. Biophys.*, Vol. 39, No. 2, 131–136, 2000.
28. Gavrieli, Y., Y. Sherman, and S. A. Ben-Sasson, "Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation," *J. Cell. Biol.*, Vol. 119, No. 3, 493–501, 1992.
29. Reed, J. C., T. Miyashita, S. Krajewski, S. Takayama, C. Aime-Sempe, S. Kitada, T. Sato, H. G. Wang, M. Harigai, M. Hanada, M. Krajewska, K. Kochel, J. Millan, and H. Kobayashi, "Bcl-2 family proteins and the regulation of programmed cell death in leukemia and lymphoma," *Cancer Treat. Res.*, Vol. 84, 31–72, 1996.
30. Oltvai, Z. N., C. L. Milliman, and S. J. Korsmeyer, "Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death," *Cell*, Vol. 74, No. 4, 609–619, 1993.
31. Bhattacharyya, A., D. Mandal, L. Lahiry, G. Sa, and T. Das, "Black tea protects immunocytes from tumor-induced apoptosis by changing Bcl-2/Bax ratio," *Cancer Lett.*, Vol. 209, No. 2, 147–154, 2004.
32. Childs, A. C., S. L. Phaneuf, A. J. Dirks, T. Phillips, and C. Leeuwenburgh, "Doxorubicin treatment in vivo causes cytochrome C release and cardiomyocyte apoptosis, as well as increased mitochondrial efficiency, superoxide dismutase activity, and Bcl-2:Bax ratio," *Cancer Res.*, Vol. 62, No. 16, 4592–4598, 2002.
33. Wei, H., B. Kang, W. Wei, G. Liang, Q. C. Meng, Y. Li, and R. G. Eckenhoff, "Isoflurane and sevoflurane affect cell survival and BCL-2/BAX ratio differently," *Brain Res.*, Vol. 1037, No. 1–2, 139–147, 2005.