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A review on combined biological effects of microwave and other physical or chemical agents

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ABSTRACT

With the development of science and technology, microwave has been used in many fields such as industry, military, medicine and communication. People are living in a lapped and dynamic electromagnetic environment. Concerns about potential hazards of microwave are getting increasing attentions. The single biological effects of microwave were widely discussed which was considered harmful. Relevant safety standards had been formulated and applied. However, the real environment was more complex. Microwave was not the only factor that organisms might be affected. Other physical or chemical factors, such as ionizing radiation (IR), ultraviolet (UV), magnetic field and chemical drugs, often existed with the microwave radiation. Even the microwave itself could create combined exposure situation, because the environment consisted different frequency microwaves. Nuclear weapons and high-power radar could produce microwave and ionizing radiation. The ultraviolet ray and magnetic field generated by the instrument's operation could act on the human bodies along with the microwaves. The combination of radiofrequency therapy and chemotherapeutic agents was also commonly used in cancer therapy. Therefore, the combined biological effects of microwaves and other physical or chemical factors were very important. This review had covered the original articles in this aspect. In order to better understand the combined biological effects, the comparative studies of different frequency microwaves were also included. Differences in biological effects were found among different frequency microwaves, and the combined biological effects contained both hazards and benefits. Findings in combined biological effects were very practical for rational uses of microwave technologies.

Keywords: *Combined biological effects, microwave, ionizing radiation, laser, ultraviolet light, drug, magnetic field.*

▶ ***Review article***

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INTRODUCTION

The earliest applications of microwaves could be tracked back to the 19th century, but the unprecedented wide applications of microwaves was in last century. In recent decades, microwave had been widely used in many fields, such as industry, military, medicine and communication. Microwave is electromagnetic wave whose wavelength is longer than that of the infrared but shorter than that of the radio wave (wavelength: 1mm~1m, frequency: 300MHz~300GHz). The potential hazards of microwaves are getting increasing attentions. Many researches about biological effects of microwaves showed that microwaves had bad influences on health. According to current studies, the injury effects mainly appeared in sensitive systems such as hematopoietic system, nervous system, circulatory system, reproductive system, endocrine system and so on(1-7). In bioelectromagnetics, specific absorption rate (SAR) was used to measure the energy absorbed by organisms. SAR was defined as the power absorbed per mass of tissue with the unit of watts per kilogram (W/kg). Most researches were conducted at a specific frequency of microwave, which didn't fully represent the biological effects of microwave.

However, the real environment was much more complex than the simulative laboratory conditions. Microwaves can be divided into many different bands and microwave exposure in the real environment is not consisted of one single frequency. Investigations on the biological effects of microwave must include cases of combined exposure of multiple frequencies.

In some specific conditions, microwave is used with other electromagnetic waves, such as IR and UV. In industry and military fields, the combined exposure of IR and microwave was very common. High voltage radio frequency transmitter contained magnetron tube and rectifier which could produce IR and microwave at the same time. Nuclear weapon could produce high fatal IR and microwave pulse when exploded. Besides, in order to get better clinically curative effect, many combined therapies were created [8]. The clinical cancer treatments contained not only chemotherapy drugs but also some new methods, for example, the microwave therapy [9]. Therefore, we had to pay attention to the combined biological effects of microwaves and chemotherapy drugs.

It was observed that the combined exposure of microwaves and multi-factors might cause combined biological effects on organisms. Therefore, researches on combined biological effects were very important. Compared with researches on single frequency microwave, the combined exposure of microwaves and other factors were of greater application prospects and significance. This review focused on the studies of combined biological effects of microwave and other physical or chemical factors, such as multi-frequency bands microwave, IR, UV, magnetic field and chemical drugs. Moreover, possible protective measures against microwaves were also discussed in this review.

Combined biological effects of multiple frequencies microwave exposure

The rapid popularization and development of communication technology made the whole world covered by kinds of communication signals. The physical characteristics of electromagnetic waves are closely related to their frequencies. According to the electromagnetic spectrum, the most common used microwaves in our daily life included the Global System for Mobile Communication signals (GSM) and Third Generation Mobile Communication signals (3G). In most parts of the world, people were living in a complex electromagnetic environment composed of microwaves with different frequencies. In addition, radar, as a radio signal launcher, was widely used to manage the territorial sky in worldwide. Parts of the radar signals belonged to microwaves and possessed large power. People developed different kinds of radar to perform various tasks. When many types of radar worked at the same time, it would form a complex electromagnetic environment. As a result, the nearby residents and workers were exposed to a complex electromagnetic environment. Concerns about potential hazards of microwave signals had attracted people's attentions and many efforts were made and proved microwave could alter healthy parameters [10-12]. However, those studies were focused on single frequency.

The potential hazards of multiple-frequency microwaves were still unknown and scholars were tried to figure out. Korean researchers had conducted series experiments with combined exposure to multiple communication signals, the code division multiple access (CDMA, 849MHz) and wideband code division multiple access (WCDMA, 1.95 GHz), which were widely used as communication signals. The animals and cultured cells were exposed to the CDMA and WCDMA simultaneously with the SAR of 2W/kg for each signal. The biological effects were evaluated through changes of biological indexes and physiological indexes and the details of these results were listed in table 1.

In a long-term exposure study, rats were exposed to CDMA and WCDMA simultaneously

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for 12 months and no significant pathological and biochemical changes was found[13]. In a study of endocrine system, rats were exposed to CDMA and WCDMA for 8 weeks and levels of thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), triiodothyronine (T3), thyroxine were examined [14]. Moreover, in the study of reproductive system, rats were exposed for 12 weeks and the sperm counting, spermatogonial cell cycle examination, apoptotic cell counting, and expression levels of tumor protein p53 (p53), B-cell lymphoma-2 (*bcl-2*), growth arrest and DNA damage-inducible genes 45 (*GADD01*), cyclin G and heat shock protein 70 (HSP70) were detected to be evaluated. However, the changes were not with statistical differences and eventually came to a negative conclusion [15].

One of the in vitro studies was about the stress response induced by microwave, the MCF10A cells were divided into 4 groups (control group, CDMA group, WCDMA group, combined exposure group) and exposed for 3 consecutive days. It was universally acknowledged that the HSP27 and ERK1/2 phosphorylation were sensitive markers of the stress responses for MCF10A cells. The phosphorylation levels of HSP27 and ERK1/2 significantly increased after single CDMA exposure and combined exposure (CDMA+WCDMA) in MCF10A cells at the 2nd and 4th hour after exposure [16].

Microwave was considered as group 2B carcinogen [17]. In Lee's study, AKR/J mice were used to detect the carcinogenic effects of combined microwave exposure for its susceptibility to lymphoma. The survival rate, lymphoma incidence rate and splenomegaly incidence rate were examined and no differences was found between control group and exposure group [18]. Moreover, the murine fetus is very sensitive to physical-chemical factors. A study detected the teratogenic effects of combined microwave exposure (CDMA and WCDMA) on pregnant mice for the whole gestation period and no significant results were found in the mortality, growth retardation, changes in head size, and other morphological abnormalities of fetuses, which indicated that the combined exposure didn't cause any obviously adverse effects on mice fetuses [19].

Negative conclusions had been drawn from studies of the CDMA and WCDMA, such as long-term effects, certain organ system effects, tumorigenicity and teratogenicity. This was good news for everyone, because it proved that our communication equipment was relatively safe for its low power. The exploration of the biological effects of microwave should further explore the combined effects of high power microwave.

In Jauchem's study, the male SD rats were divided to 4 groups (control, 1GHz group, 10GHz group, combined group) and the whole body average SARs were controlled equivalently at 12W/kg in each exposure group. All animals were killed by high power microwave and the average survival times were recorded. Animals in 1GHz group died first then the combined group and the 10GHz group last. Compared with single frequency exposure groups, no unusual physiological responses were found in multiple frequency exposure group [20].

Under experimental conditions of same power, no significant differences were found between single frequency and multi-frequency exposure, which suggested that the microwave effects mainly depended on its power. However, we couldn't deny the fact that microwave characteristics were frequency-dependent. The similarity of frequencies used in this experiment might be one of reasons for the negative conclusion. We should not stop exploring the combined biological effects of microwave.

Comparative studies of biological effects of microwaves

As mentioned above, researches about biological effects mainly focused on single frequency microwave exposure. In these studies, some studies had discussed the differences of biological effects among microwaves of same power but different frequencies. Studies in this aspect were considered to be helpful in the exploration of combined biological effects. The comparative studies were involved in this part. (The details of each article we referenced in this review were listed in table 2)

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Table 1. Combined biological effects of different frequency microwave exposure.

Author	Research Project	Frequency	Exposure	SAR (W/kg)	Experimental Model	Detection Index	Result
Jin *et al.*, 2011 (13)	Long-term effect	CDMA:849MHz WCDMA: 1.95GHz	45min/d,5d/w, 12months	CDMA: 2 WCDMA: 2	SD rats	Blood routine，pathological examination， blood biochemistry	Unchanged
Jin *et al.*, 2013 (14)	Endocrine system	CDMA:849MHz WCDMA: 1.95GHz	45min/d,5d/w,2 months	CDMA: 2 WCDMA: 2	SD rats	TSH、T3、T4、ACTH、sex hormone	Unchanged
Lee et al., 2012(15)	Reproductive system	CDMA:849MHz WCDMA: 1.95GHz	45min/d,5d/w,3 months	CDMA: 2 WCDMA: 2	SD rats	Sperm counts、spermatogonium cycle、apoptotic cell counts、P53、Bcl-2、GADD45、Cyclin G、HSP70	Unchanged
Kim *et al.*, 2012 (16)	Stress reaction	CDMA:849mhz WCDMA: 1.95Ghz	2h simultaneous exposure	CDMA: 2 WCDMA: 2	MCF10A cell line	HSP27 ERK1/2 phosphorylation	Significant stress response
Lee *et al.*, 2011 (18)	Tumorigenicity	CDMA:849MHz WCDMA: 1.95GHz	45min/d, 5d/w，42weeks	CDMA: 2 W/kg WCDMA: 2 W/kg	AKR/J mice	Survival rate、morbidity (lymphoma)	Unchanged
Lee *et al.*, 2009(19)	Teratogenesis	CDMA:849MHz WCDMA: 1.95GHz	Gestation period exposure	CDMA: 2 WCDMA: 2	Pregnant mice	Embryonic mortality、stunting prevalence、morphologic changes in embryo	Unchanged
Jauchem *et al.*, 2000 (20)	Lethal effect	HPM: 1GHz & 10GHz	Exposed simultaneously and record survival time	1GHz: 6 10GHz: 6	SD rats	Average survival time	Negative

Differences in biological effects of different frequency microwave

So far, both of S-band and X-band high power microwaves (HPM) have been widely used microwave, and the electromagnetic pulses (EMP) also have become a common electromagnetic pollution. A series of studies compared the differences in the biological effects of the three common electromagnetic waves, S, X-band HPM and EMP with same parameters: S-HPM, 2.856GHz, 100mW/cm^2; X-band HPM, 9.8GHz; EMP, 6×104V/m. The three kinds of electromagnetic waves could lead to the decline of learning and memory, but the degrees were not the same. Scholars used the Morris water maze to evaluate the spatial learning and memory ability of rats and the changes in average escape latency (AEL) were recorded. After microwave exposure, AEL of the exposure groups prolonged than that of the control group, which indicated the adverse effects on spatial learning and memory ability. Besides, changes in S, X-HPM groups were more obvious than EMP [21]. The hippocampus structures were also observed through lighting microscope (LM) and electron microscope (EM). At the 6 hour after exposure, ischemic changes appeared in ultrastructure of hippocampal neurons. At the 3 day after exposure, vacuoles, secondary lysosomes and widened vascular gaps were observed. The degree of injury ranking from serious to light was: X-HPM group,

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S-HPM group and EMP group [21]. The anal temperatures were also detected, and the sequence ranked from serious to light was: X-HPM group, S-HPM group and EMP group. Generally, the injury effects were consistent with thermal effects.

The effects of S, X-band HPM and EMP on the immune system were also different. In the study of the immune system, Wang *et al.* [22] used S, X-HPM and EMP to irradiate rats (Wistar, male) for 20 minutes. The injury degree of spleen was evaluated by histological and ultrastructure examinations and the injury ranked from serious to light was: X-HPM group, S-HPM group and EMP group.

The relationship between injury degree and the electromagnetic environment in reproductive system was different from that in hippocampus and immune system. In the study of reproductive system, rats (Wistar, male) were sacrificed for pathological examinations after S, X-HPM and EMP exposure for 20 minutes. The pathological examinations included testis weighing, LM observation and seminiferous tubules quantitative lesion analysis. The EMP group showed the most serious injury, followed by the X-HPM group and S-HPM group [23]. Therefore, we had reasons to believe that the various organs might have different sensitive frequencies.

According to the temperature changes, injuries induced by electromagnetic exposure in most organs were consistent with the power as well as the thermal effects, indicating the thermal effects might play an important role in the injury effects. However, the injuries of S, X-band HPM and EMP were not same in different organs or systems, which indicated that the non-thermal effects could not be ignored.

Studies in this aspect had some human trials. Adair *et al.* [24] measured thermoregulatory responses of heat production and heat loss in two different groups of 7 adult volunteers (males and females) during 45min dorsal exposure of the whole body to 450/2450 MHz continuous wave. Exposure groups were controlled at the same SARs of 15.4W/Kg for comparable analysis, and no changes in metabolic heat production occurred under any exposure conditions at either frequency.

Differences in mechanisms of different frequency microwave

Like the differences in effects, the comparative studies were also found the differences in injury mechanisms among S, X-HPM and EMP. Wang et al. [25] used Raji cells (lymphoma cells) as experimental materials to study the expressions of cysteinyl aspartate specific proteinase 3 (caspase-3) and cysteinyl aspartate specific proteinase 8 (caspase-8) after MW exposure. In this study, 4 groups were prepared: control group, S-HPM group, X-HPM group and EMP group. After exposure, caspase-3 was found up-regulated in all exposure groups, but the caspase-8 only up-regulated in the X-HPM group while down-regulated in the other exposure groups, suggesting existence of differences in mechanisms. These studies indicated microwave of different frequencies might be different in mechanisms.

As we all known, the B-cell lymphoma-2 gene (Bcl-2) and Bcl-2 associated X Protein (BAX) are important members of the Bcl-2 apoptotic regulating gene family. Scholars had conducted researches and found that the expressions of Bcl -2 and BAX were different in different tissues after MW exposure. Dou et al. [26] detected the changes of Bcl-2 and Bax expression in rats' myocardium after S-HPM, X-HPM and EMP exposure and found that the expression levels of Bcl-2 and Bax were up-regulated, indicated the two genes might play roles in the injury effects. However, another study detected the expressions of Bcl-2 and Bax in spleen tissue after S-HPM, X-HPM and EMP exposure, no statistically changes were found[27]. Those results suggested that injury mechanisms might be different between myocardial tissue and spleen tissue.

In hippocampus, injury mechanisms of different electromagnetic environments were discussed. Zuo *et al.* [28] studied the mechanisms of hippocampus injury in rats caused by S-HPM, X-HPM and EMP. The changes of Raf/MEK/ERK (MAPK signaling pathway) were detected, but results were similar between each group. Another research studied the molecular

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mechanisms in hippocampal injuries. Western bolting was used to detect the expression of injury-related factors, such as glial fibrillary acidic protein (GFAP) and interleukin-1β (IL-1β). The expression of GFAP was down-regulated, while expression of IL-1β was up-regulated, and the injury degree ranked from the most serious to the lightest was EMP, X-HPM and S-HPM (29). According to the research in hippocampus, changes in molecular mechanisms could well explain the injury effects. The results of this section showed that the results were partially supported by mechanisms, which laid a foundation for preventing microwave damage and understanding the combined biological effects.

No differences between continuous wave (CW) and pulsed wave (PW)

There were comparative researches on the biological effects between continuous wave (CW) and pulsed wave (PW). Volunteers (two females, five males) were exposed to 2450 MHz CW and PW and measured thermophysiological responses of heat production and heat loss in 30 minutes. The results showed that the local sweat rate and skin blood flow depended on temperatures and SARs, but did not depend on the modulation style of microwave (30). Cytological experiment came to the same conclusion. Pakhomov et al. (31) compared biological effects of CW (9.3GHz) and extremely high power pulses (EHPP, 9.3GHz) at the same carrier frequency and average power. The SARs in surface was 3.2kW/Kg and 0.6mW/kg in 24mm depth. Yeast cells density was determined by nephelometry. Compared to unexposed control groups, no statistical differences were found between CW and PW groups.

Table 2. Comparative Studies of Biological effects of Different Parameter microwave

Author	Research Project	Microwave Parameter	Experimental Animal	Detection Index	Injury Degree
Chen J, 2006 (21)	learning and memory ability	S- HPM: 100mW/cm^2 L- HPM: 100mW/cm^2 EMP: 6×10^4V/m	Wistar rats (male)	Morris water maze (AEL)	AEL: HPM＞EMP Anal temperature: X-HPM＞S-HPM＞EMP
	Hippocampus structure	S- HPM: 100mW/cm^2 L- HPM: 100mW/cm^2 EMP: 6×10^4V/m	Wistar rats (male)	HE staining, LM, EM	X-HPM＞S-HPM＞EMP
Yao H, 2010 (23)	Testis structure	S- HPM: 100mW/cm^2 L- HPM: 100mW/cm^2 EMP: 6×10^4V/m	Wistar rats (male)	LM, weighting, seminiferous tubules quantitative lesion analysis	EMP＞X-HPM＞S-HPM
Wang W, 2008 (22)	Spleen structure	S- HPM: 100mW/cm^2 L- HPM: 100mW/cm^2 EMP: 6×10^4V/m	Wistar rats (male)	HE staining, LM, EM	X-HPM＞S-HPM＞EMP
Wang W, 2012 (25)	Lymphoma cells	S- HPM: 100mW/cm^2 L- HPM: 100mW/cm^2 EMP: 6×10^4V/m	Raji cell	Caspase-3，8	S, X-HPM & EMP：Caspase-3 ↑；X-HPM: Caspase-8 ↑ S-HPM & EMP: Caspase-8 ↓
Zuo HY, 2006 (28)	Nerve injury factors (hippocampus)	S- HPM: 100mW/cm^2 L- HPM: 100mW/cm^2 EMP: 6×10^4V/m	Wistar rats (male)	GFAP，IL-1β	GFAP↑IL-1β↓change degree：X-HPM＞S-HPM＞EMP
Zuo HY, 2009 (28)	MAPK pathway (hippocampus)	S- HPM: 100mW/cm^2 L- HPM: 100mW/cm^2 EMP: 6×10^4V/m	Wistar rats (male)	Raf/MEK/ERK	Unchanged
Dou Y, 2010 (26)	Bcl-2 apoptotic regulating gene family (myocardium)	S- HPM: 100mW/cm^2 L- HPM: 100mW/cm^2 EMP: 6×10^4V/m	Wistar rats (male)	Bcl-2，BAX	X-HPM＞S-HPM＞EMP
Wang W, 2014 (27)	Bcl-2 apoptotic regulating gene family (spleen)	S- HPM: 100mW/cm^2 L- HPM: 100mW/cm^2 EMP: 6×10^4V/m	Wistar rats (male)	Bcl-2，BAX	Unchanged
Adair et al., 2001 (24)	Thermoregulatory responses	CW:2450 MHz PW:9.3GHz	7adult volunteers (males and females)	Heat production and heat loss	Unchanged

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Combined biological effects of microwave and IR

IR, electromagnetic or corpuscular radiation, is able to cause ionization and its health hazard is generally accepted. The use of IR was most commonly seen in radiotherapy. Recently, clinical treatments of tumors often applied radiotherapy and microwave therapy simultaneously for better curative effect. Besides, both microwave and IR are injury factors of nuclear devices. High voltage radio frequency transmitter which contains magnetron tube and rectifier could produce IR and microwave when working. Therefore, the combined biological effects of microwave and IR are common and important. Since 1960s, researches about combined biological effects of microwave and IR had been carried out in various fields, such as occupational health, radioactive medicine and public health. In decades, people had found different results in experiments and the results could generally be divided into synergetic effects and antagonistic effects. In this part, we had reviewed the main research findings of combined biological effects of microwave and IR.

Synergetic effects of microwave and IR

Some animal experiments found that the combined exposure of IR and microwave could cause more serious injury than single IR exposure and this phenomenon was described as synergetic effect. The situation of combined injury was similar to radiation injury, suggested that the injury was mainly caused by IR. (Details of the researches of synergetic effects were listed in table 3).

Table 3. Synergetic effects of microwave and IR

Author	Microwave	IR	Exposure Method	Experimental Animal	Detection Index	Result
Jia YF, 2006 (32)	Microwave ($50mW/cm^2$, S-band)	γ-ray (5.5Gy)	γ-ray exposure before Microwave exposure, 5min	Mice	Histology, ultrastructure of bone marrow and peripheral blood	MW could aggravate γ-ray damage effects
Thomson RA, 1961 (33)	Microwave ($165mW/cm^2$)	X-ray (7.5Gy)	4days X-ray exposure followed 120min MW exposure	Dogs	Mortality	X-ray group: 30% combined group: 70%
Cao *et al.*, 2009 (34)	Microwave (900MHz 2, 4, $6W/m^2$)	γ-ray (1Gy)	Microwave exposure followed γ-ray exposure	HSG cells	ROS & HSP70	ROS(↑) HSP70(-) MW could aggravate the changes of γ-ray
Zhang W, 2008 (35)	Microwave (900MHz; 0, 20, 40, $60W/m^2$)	γ-ray (5Gy)	Microwave exposure (2h/d, 3d) followed γ-ray exposure	Human glioma cells	Cell proliferative activity and colony formation rate	Microwave could aggravate γ-ray damage effects, and the effects positively related to power of MW

The synergetic effects had been proved in many in vivo studies. A research used mice as experimental model to study the influence of combined exposure of S-band microwave ($50mW/cm^2$) and γ-ray (5.5Gy) on bone marrow cell proliferation. Microstructure, ultrastructure and peripheral blood were detected to evaluate the injury degree. They found that the damage effects were mainly caused by γ-ray, and microwave would aggravate the injury effects [32]. Thomson *et al.* [33] radiated dogs with X-ray (7.5Gy) for 4 days, then radiated dogs with microwave ($165mW/cm^2$) for 120 minutes. The mortalities of dogs in different groups (control group, microwave group, X-ray group, and combined group) were calculated. They found

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that the mortality of combined exposure group increased from 30% to 70% compared with the X-ray group.

In vitro studies also found synergistic effects. A research used SHG cells as experimental material to study the stress responses. Cells were radiated by microwave (900MHz) and γ-ray (8Gy). As we all known, HSP70 and ROS are sensitive parameters of cell stress responses. The ROS were up-regulated in both γ-ray group and combined group, and changes in combined group were slightly obvious than γ-ray group. They considered the changes were mostly caused by γ-ray, and microwave enhanced it. But no significant changes in HSP70 were found in protein or mRNA levels [(34)]. Another study used human glioma cells to study the combined effects on cell proliferation activity of microwave (900MHz) and γ-ray (5Gy) exposure. Glioma cells were randomly divided into control group ($0W/m^2$), low dose group ($20W/m^2$), middle dose group ($40W/m^2$) and high dose group ($60W/m^2$) based on the power density of microwave. Each group was exposed to microwave first, and then radiated by γ-ray. Cell proliferative activity and colony formation rate were detected. Results showed that the cell proliferative activity and colony formation rate decreased in exposure groups compared to control group. And the injury degree increased with the microwave power density. The results suggested that microwave and γ-ray had synergistic effect, which was related with the dose of microwave [(35)]. To sum up, both in vivo and in vitro studies were included in this part. This series of studies demonstrated the aggravating effects of microwave on IR injuries. Besides, IR was dominant in the combined injury effects.

Antagonistic effects of microwave and IR

Some researchers hold opposite opinions on the combined biological effects between microwave and IR. They found that the microwave could relieve IR injuries and described this founding as antagonistic effects. (Details of those antagonistic effects researches were listed in table 4).

Previous microwave radiation could prolong the survival time and reduce the mortality of experimental dogs exposed to IR. Michaelson *et al.* [(36)] used microwave ($100\sim165mW/cm^2$, 2.8GHz) radiated dogs for 30~360 minutes first and then followed by the X-ray (3.6Gy). Compared to the X-ray group, the 60th day mortality of combined group declined from 68% to 17~40%. Another study used X-ray (9.5Gy) radiated abdomen of experimental dogs, the 4th day mortality of X-ray exposure group was 37.5%, whereas no dog was dead in another group that previously irradiated by microwave (PW, 100mW/cm2). When radiated dogs with X-ray (2.5Gy), result showed that the previous exposure of microwave could extend the average survival time from 22h to 43h. According to the results, animals pretreated with microwaves were less sensitive to IR than those did not pretreated with microwaves [(37)].

In this part, the listed researches had shown that microwave pretreatment might help relieve IR induced injuries and this provided a new idea for the protection of ionizing radiation.

Complexity of combined effects between microwave and IR

The combined effects between microwave and IR were controversial. Most scholars considered that combined effects between

Table 4. Antagonistic effects of microwave and IR.

Author	Microwave	IR	Exposure Method	Experimental Animal	Detection Index	Result
Michaelson SM, 1961 [(36)]	MW ($100\sim165mW/cm^2$ 2.8GHz)	X-ray (3.6Gy)	MW exposure before X-ray exposure	Dogs	60^{th} day mortality	Single X-ray group: 68% combined group: 17-40%
Michelson SM, 1962 [(37)]	MW	X-ray (9.5Gy)	MW exposure before abdominal X-ray exposure	Dogs	4^{th} day mortality	Previous exposure of MW could extend the average survival time from 22h to 43h

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microwave and IR were closely related to the exposure sequence and exposure time. If microwave was irradiated during IR's acute injury stage, it would aggravate the injury. Oppositely, previous exposure of microwave could relieve IR's injury effects. (Details of the complexity researches were listed in table 5).

The order of IR and microwave exposure would affect the combined biological effects. Thomson et al. [38] used X-ray (7.5Gy, 1h/d, and 30d) radiated mice firstly, and the mice were dead in 44 days. The average survival time was 19 days. When the mice were irradiated by microwave (10mW/cm2, 2.8GHz, 1h/d, 30day) after X-ray irradiation, then the average survival time decreased to 13 days, indicated that the injuries were aggravated by the later microwave exposure. When mice were radiated microwave (1h/d, 2.8GHz, 3day) before X-ray irradiation, then the average survival time increased 7 days with the 44th day mortality reduced from 100% to 67%. The exposure sequence and moment of microwave were considered decisive factors in combined biological effects of microwave and IR.

Combined biological effects of microwave and other physical factors

Combined biological effects of microwave and magnetic field

In the modern times, the magnetic field is no longer strange as a new environmental factor. Applications of a magnetic field in medical treatment aroused people's attentions and different magnetic fields were used to treat diseases. Scholars found that magnetic field might relieve microwave induced injuries. Lai *et al.* [39] studied the combined biological effects of microwave and pulsed magnetic field on spatial learning and memory abilities. The mice were radiated by microwave (2450MHz, 2mW/cm^2) and/or pulsed magnetic field (60mG) for 1 hour. In this study, 4 groups were prepared: control group, microwave group, magnetic field group and combined group. Results showed that microwave could prolonged the AEL of mice, indicating the spatial learning and memory ability was damaged. AELs in combined group were shorter than those in the microwave group, which indicated that the pulsed magnetic field could relieve the microwave induced spatial learning deficits. Mechanism of this combined biological effects were not clear, one of the possible hypothesis was that the microwave transfer might be interfered by the magnetic field.

Combined biological effects of microwave and UV

The combined exposure of microwave and UV was most common, because both microwave and UV covered the earth. However, in academic researches and special uses, the power of microwave and UV were much higher than natural conditions. In this part, we focused on the combined biological effects of high power microwave and UV.

Table 5. Complexity of combined effects between microwave and IR.

Author	Microwave	IR	Exposure Method	Experimental Animal	Detection Index	Result
Thomson et al., 1965 [38]	Microwave (10mW/cm^2, 2.8GHz)	X-ray (7.5Gy)	Single X-ray exposure	Mice	44th day mortality & average survival time	44th day mortality: 100% average survival time: 19d
			Microwave exposure after X-ray exposure			44th day mortality: 100% average survival time: 13d
			Microwave exposure before X-ray exposure			44th day mortality: 67% average survival time: 26d

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Microwave and UV could be used simultaneously to improve sterilization efficiency. One research studied the synergistic bactericidal effects of microwave (2450MHz, 500W) and UV (254nm, 8W). Microwave and UV were used to radiate bacterial culture. Samples were taken at 2 minutes, 6 minutes and 10 minutes after irradiation for colony counting and sterilization rate calculating. Results showed that the combined group had better bactericidal effects than other groups (40).

The response of the organism to the combined exposure of microwave and UV was also stronger. Another research studied the combined biological effects of UVA (365nm, 20W), UVC (254nm, 30W) and microwave (2450MHz, 10mW/cm2). SD rats were randomly divided into 6 groups: control group, UVA group, UVC group, microwave group, UVA + microwave group and UVC + microwave group. Blood samples were collected for examination at the 14th day after radiation, and the expression levels of prostaglandin E2 (PGE2) and cyclic adenosine monophosphate (cAMP) increased. Changes in combined groups were more significant than other groups, indicated the existence of synergistic effects, but no difference were found in biological effects between UVA and UVC (41). According to the articles we reviewed, the combined biological effects of high power microwave and UV existed, and there were synergistic effects between the two factors.

Combined biological effects of microwave and chemical drugs

Combined biological effects of microwave and antitumor drugs

Radiotherapy and chemotherapy are common methods in clinical tumor treatments. Many newly developed microwave technologies were used in medical treatment in recent years, such as microwave therapy and microwave ablation. In the treatment of tumor, radiotherapy and chemical therapy were two major methods. The combined biological effects of IR and microwave had been discussed above. In this part, we were going to discuss the combined biological effects of microwave and antitumor chemical drugs. According to the microwave induced changes in curative effects, the kinds of combined biological effects could be summarized into 3 kinds: accelerating effects, inhibiting effects and non-effects.

Some studies have shown that microwave could enhance the tumor cytotoxicity of anticancer drugs. Wang *et al.* (42) used healthy human lymph cells as experimental material and the cells were treated with mitomycin C (MMC) and bleomycin (BLM) respectively. Exposure groups were irradiated by microwave (1.8GHz, 3W/Kg). The DNA damages were detected to evaluate the biological effects of drugs and microwave. Results showed that cytotoxicity of MMC was enhanced by microwave; indicated microwave could help MMC get better curative effects for clinical uses. Another study used millimeter wave (61.22GHz, 31mW/cm^2) and cyclophosphamide (CPA) treated mice. After the comparison and analysis of results in microwave + CPA group and CPA group, scholars found that the microwave exposure could accelerate the expression of tumor necrosis factor- α (TNF- α) and interferon γ (IFN-γ) in T cells, suggested the existence of accelerate effects between MW and CPA (43). The accelerating effects were very meaningful for tumor patients, but the mechanisms were not clear. More efforts should be put in this aspect.

Another study reported inhibiting effects of microwave and antitumor drugs. HL60 cells in the combined group were treated by microwave (900MHz, 12mW/cm^2) and Adriamycin, and the proliferation activity of the combined group increased compared with single Adriamycin group. Results suggested that the microwave may inhibit cytotoxicity of Adriamycin, which resulted in the increase of proliferation activity in HL60 cells (44).

In addition, some studies suggested there were no interactions between microwave and antitumor drugs. Maes *et al.*(45), treated human whole blood with microwave (935.2MHz, 0.3~0.4W/Kg) and MMC, then performed the chromosomal aberrations test, but no significant differences were found between experimental groups and control group.

In summary, there were different conclusions

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about the combined effects of microwave and antitumor drugs, but the scholars' efforts were valuable and meaningful. The laws of combined effects between microwave and chemotherapy drugs would provide new ideas for clinical therapy.

Combined biological effects of microwave and psychotropic drugs

Nervous system was considered to be one of the most sensitive organs for microware radiation. When using phones, heads were closely exposed to phone signals. With the wide use of the handheld communications devices, some scholars were worried about the influences of microwave on nervous system, as well as the psychotropic drugs.

Some studies in combined biological effects of microwave and drugs were carried out. Currently, the researches in this field mostly focused on effects testing. A research used rats as experimental model to study the combined biological effects of microwave (2.8GHz, 1mW/ cm^2) and nervous drugs (diazepam, and chlorpromazine respectively). The effects were measured on a fixed interval schedule of food reinforcement with rats. Results showed that the combination of microwave and drugs did not produce any alterations compared with control group[46]. In addition, another study recruited 25 right-handed healthy male college students to study the combined biological effects of caffeine and 3G signals (0.73W/kg) on nervous system by the electroencephalography (EEG), reaction time and P300 event related potentials (ERP). However, no significant differences were found between the combined group and caffeine group [47]. From this part, the present studies came to negative results and the researches could not draw a convinced conclusion. The existence of combined effects was not clear and studies in this field were not enough to answer the question.

Combined biological effects of microwave and other drugs

There were also researches focused on the combined biological effects of microwave and other drugs. A study used frog's separated myocardium to study the combined biological effects of caffeine (1mM) and microwave (915MHz, 8~10W/kg, 40min), the contraction rate and range in the combined group increased more than those of the caffeine group. The differences between each groups were significant in statics, suggested the existence of interaction between MW and caffeine[48]. However, many tries found the negative results. Brescia *et al.*[49], used cultured mammalian cells as experimental models to study the combined biological effects of microwave (1950MHz, 0.5 & 2.0W/kg) and ferrous ion. Flow cytometry method (FCM) was used to detected reactive oxygen species (ROS), which was considered as a sensitive parameter of oxidative stress. Under these experimental conditions, no combined biological effects were found.

All of those studies should be paid attentions. The combined biological effects of microwave and drugs were closely related to people's health. Studies in the aspect were needed.

Protections of combined biological effects of microwave and multiple factors

According to the above context, parts of the combined biological effects of multiple-frequency microwave, microwave and IR, UV, chemical drugs were harmful. The injury effects of microwave had been gradually revealed and the protection methods should be paid more and more attentions. Lots of researches had been performed in this field, which contributed to the protection of harmful combined biological effects.

Physical shielding was the best way to avoid microwave hazards, the physical screening functions of protective clothes and equipment had been recognized by lots of people[50], and other new types of microwave screening materials had also been developed[51]. For people who worked in the microwave exposure environment, shielding devices and fortifications could be used to ensure personal security. It could prevent the generation of combined biological effects.

Moreover, there were anti-microwave drugs for prevention and treatment. Studies found that some botanicals had significant curative effects

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for microwave-induced injuries, such as Kang Fu Ling, An Duo Lin and flaxseed oil [52-54]. Those drugs could be used to treat the injury caused by microwave, but the pesticide effects in combined injury were not clear.

In summary, the protections of combined injuries of microwave and multiple factors are far from completed. There were no general standards for the protection of combined injuries and were no sensitive indicators for combined injuries, which was a big problem for injury diagnosis. Researches about protections for combined injuries should be carried out in the future.

CONCLUSION

Researches on combined biological effects of microwave and multiple factors were aimed at solving the problems of environmental health and making better use of microwave. Scholars had simulated the combined exposure environment and tested the possible biological effects on different organs and systems. The researches about combined biological effects of multiple frequencies microwave were mostly focused on the communication frequency, whose powers were mainly in low level. The long-term effects were also included. So far, the communication microwave signals have been basically considered to be safe. The combined biological effects of high power microwave did not get enough attention and there were few researches at present. But studies of single frequency microwave had proved the hazards of microwave in different organs and tissues [55-58].

In comparative studies among microwaves with different frequencies, we found that the injury degree was closely related with the exposure frequency. However, the sensitive frequency of different tissues and organs might be different and there were also differences in the molecular mechanism. The injuries effects were mainly caused by thermal effects and closely associated with the absorptive capacity [59]. Besides, no significant differences were found in comparative studies of thermal effects between PW and CW. Studies of the injury laws in this aspect were relatively clear.

As for the combined biological effects of microwave and IR, the majority believed that the microwave and IR had synergistic effects, while minority hold the opposite view and they believed that there was antagonistic effects. Possible explanations for antagonistic effects were that the previous microwave treatment could make animals be more tolerable to IR. Experimental conditions of studies in this field were various, such as microwave frequency, power, exposure time, exposure method and experimental animals. Therefore, up to now, no confirm conclusions had been concluded.

Purposes of researches of combined biological effects of microwave and other physical factors (UV, magnetic field) and chemical drugs were to figure out whether combined effects were different from single effects. Most of the researches mentioned in this part were applicable studies. The combined effects of microwave and antitumor drugs had three kinds: accelerating effects, inhibiting effects and non-effects. Those findings had great potential medical value. If we could made good use of those findings, the curative effects of antitumor drugs would be controlled.

With the development of microwave technology and science, new kinds of combined biological effects of microwave and other physical or chemical factor will occur. All efforts made in this field were aimed to build a healthier world.

PROSPECT

In summary, the studies of combined biological effects of microwave and multiple factors were focused on several aspects, such as multiple frequencies microwave, microwave and IR, microwave and magnetic field, microwave and UV, microwave and drugs. By now, most studies had confirmed the existence of combined biological effects, but failed to draw firm and unanimous conclusions. There were still many unsolved problems: 1. The experimental

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conditions in different papers were different, which made the results to be incomparable; 2. The researches remained in exploration of existence of combined effects, there were blanks in does-effect relationship research as well as mechanism research; 3. Many signaling pathways and molecules had been confirmed to participate in the injury process, but no sensitive biological indicators were found; 4. The diagnosis and protective measures for combined effects was incomplete.

In short, lots of beneficial explorations had been performed for combined biological effects of MW and multi-factors, but lots of efforts still should be made in this field.

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