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A Review of Hydrogen as a New Medical Therapy

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ABBREVIATIONS: Ischemia-Reperfusion (I/R); Reactive Oxygen Species (ROS); Interleukin (IL); Superoxide Dismutase (SOD); Catalase (CAT); Glutathione (GSH); Malondialdehyde (MDA); 4-Hydroxynonenal (4HNE); 8-Hydroxydeoxyguanosine (8-OHdG); Myeloperoxidase (MPO); 8-oxoguanine (8-oxoG); 8-isoprostane (8-isoPGF2α); Diamine Oxidase (DAO); Thiobarbituric Acid Reactive Substances (TBARs); Reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH); Tumor Necrosis Factor α (TNF-α); High-Mobility Group Box 1 (HMGB1); Proliferating Cell Nuclear Antigen (PCNA); Interferon-γ (IFN-γ); Chemokine (C-C motif) Ligand 2 (CCL2); Intercellular Adhesion Molecule-1 (ICAM-1); Inducible Nitric Oxide Synthase (iNOS); Ionized Calcium Binding Adaptor Molecule 1 (Iba1); Phosphorylated Lyn Tyrosine Kinase (Lyn-P); Mitogen-Activated Protein Kinase (MAPK); MAP-Extracellular Signal Regulated Protein Kinase-1 (MEK-1); Nuclear Factor-κβ (NF-κβ); Terminal Deoxynucleotidyl Transferase Deoxyuridine Triphosphate Nick-End Labeling (TUNEL).

SUMMARY

In the past few years many initial and subsequent clinical studies have demonstrated that hydrogen can act as an important physiological regulatory factor to cells and organs on the antioxidant, anti-inflammatory, anti-apoptotic and other protective effects. So far several delivery methods applied in these studies have proved to be available and convenient, including inhalation, drinking hydrogen-dissolved water and injection with hydrogen-saturated saline. This study reviews recent studies on the protectiveness of hydrogen and discusses the possible mechanisms including antioxidant ability as a gaseous signaling molecule, anti-cancer capability and others. It also tries to reveal whether endogenous hydrogen has an important role in the protective system. Nevertheless, there are still many remaining questions in the domain of hydrogen medicine and much work needs to be carried out in the future.

INTRODUCTION

A few decades ago a mysterious event made a small town called Nordenau in Germany to be world-famous. When local people used the water from a spring in a superseded mine, their illnesses, such as diabetes, tumors, gastritis and enteritis, took a turn for the better; they called this miracle the “Nordenau Phenomenon”. A Japanese doctor, Dr. George Tseng, went to that town and studied the water. He found that the water was abundant in hydrogen. This was not only true for Nordenau water in Germany, but also Hita Tenryosui water in Japan and Tlacote water in Mexico which had the same effectiveness. In fact, early in 1975, Dole found that inhalation of hyperbaric hydrogen caused a marked regression of skin tumors (1). The effective component was the hydrogen molecule in this “magical water” and in the inhalation of hydrogen. Hydrogen is a colorless, odorless, non-metallic, tasteless and highly flammable diatomic gas and it is also the lightest and most abundant chemical element which provides the source of energy for the sun by nuclear fusion to produce helium. Ohsawa *et al.* (2) discovered that hydrogen gas has the ability to protect the brain against ischemia-reperfusion (I/R) injury, through selectively eliminating the toxic oxygen radicals. So far, a lot of experiments have been carried out to confirm the properties of hydrogen including selective antioxidant properties, exerting anti-apoptotic, anti-inflammatory, anti-allergy, anti-cancer effects and so on. Reactive oxidant species (ROS) are produced in a many physiological and pathological processes and they are involved in caducity and the pathology of many diseases, including cancer, cardiovascular, inflammatory and degenerative diseases. Hydrogen selectively reduces hydroxyl radicals and peroxynitrite, especially hydroxyl radicals which are the strongest of the oxidant species, react indiscriminately with nucleic acids, lipids and proteins and there is no known endogenous detoxification system for them in the human body. So hydrogen selective reduction has very important value. Another possible mechanism underlying the cellular protection afforded by hydrogen may be an increase in antioxidant enzymes such as catalase, superoxide dismutase or heme oxygenase-1 (3,4). In addition, the anti-apoptotic properties of hydrogen *via* inhibition of caspase-3 activation have also been postulated (5). Hydrogen exhibits

anti-inflammatory activity in various injury models. Typically, oxidative stress-induced inflammatory tissue injury is inhibited by hydrogen with down-regulation of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, chemokine (CCmotif) ligand 2 and tumor necrosis factor-α (6,7). More recent studies show that hydrogen can act as a gaseous signaling molecule like nitric oxide, which can explain some unresolved questions, expanding the depth and extent of the investigation of hydrogen (8).

Delivery of hydrogen

Inhalation

Hydrogen can be easily delivered *via* inhalation with delivering the gas through a ventilator circuit, facemask or nasal cannula. There is no risk of explosion for hydrogen in air and in pure oxygen when the concentration is less than 4.7% (9). However, safety is still a common concern and the desired concentration of hydrogen must be monitored and maintained with proven, commercially available tools. The safety of hydrogen for humans is demonstrated by its application in Hydreliox, an exotic, breathing gas mixture of 49% hydrogen, 50% helium and 1% oxygen (10).

Oral intake of hydrogen-rich water

Solubilized hydrogen may be beneficial since it is a simple, convenient and safe means of delivering molecular hydrogen (11). Drinking hydrogen-rich water (HW) has the same effects (12). There are some methods to produce the HW, including dissolving electrolyzed hydrogen into pure water and dissolving hydrogen into water under high pressure. Recently a new delivery method has been invented by using the coral calcium hydride which can produce hydrogen conspicuously and it can also show its antioxidant ability (13).

Injectable hydrogen-rich fluid

Even though oral administration is safe and convenient, it cannot guarantee the concentration of hydrogen in water over time because of its volatilization and loss in

the stomach or intestine. Administration of hydrogen *via* injection may make the delivery more accurate (14).We can also use the intraperitoneal injection way, which is proved to be available.

Recent research of hydrogen *via* various disease models

Here we list the different studies based on kinds of disease models and collect all the measurement indicators to identify the properties of hydrogen.

The indicators used in the experiments include anti-oxidase markers including SOD, CAT, GSH, all show an increase. The markers of oxidative stress, like MDA, 4HNE, 8-OHdG, MPO, 8-oxoG, 8-oxoguanine, 8-isoPGF2α, DAO, TBARs, NADPH, show obvious decline. The markers of inflammation factors containing IL-1β, IL-6, TNF-α, HMGB1, PCNA, IFN-γ, CCL2, ICAM-1, iNOS, IL-12, Iba1, all drop. The marker of inflammation signals, as MAPK, MEK-1, Lyn-P, NF-κβ decrease. The markers of apoptosis, TUNEL, Annexin V, Caspase-3, Caspase-12 also display a decrease.

Hydrogen acts as a kind of antioxidant

The main property of hydrogen as a kind of antioxidant is the most basic theory in this field. At the same time, it also affects inflammation. Hydrogen can suppress the tissue-destructive production involving pro-inflammatory cytokines, TNF-a and IFN from the activated lymphocytes. Moreover, the oxidation products ROS can activate TNF-a expression by up-regulation of the NF-kB signaling pathway (15) while TNF-a can activate NADPH-oxygenase (NOX) expression that generates ROS from NADPH (16). Thus, both inflammation and oxidation processes are reciprocally related. Oxidative stress is characteristic of many processes including ischemia-reperfusion (I/R) injury, inflammatory disorders, cancer, cardiovascular and neurological diseases and aging (17). Oxidative stress is generally associated with the production of highly reactive free radicals like the reactive oxygen species (ROS). For example, excessive ROS can result in DNA fragment, lipid peroxidation and inactivation of proteins which can initiate apoptosis or necrosis. The most acceptable mechanism of hydrogen having such magical effects is that the hydrogen can electively and directly scavenge

cytotoxic oxygen radicals, especially the hydroxyl radical-the most cytotoxic of reactive oxygen species (ROS) and at the same time not react with other ROS, including O_2- and H_2O_2 since they both possess important physiological roles as signaling molecules that are involved in numerous signal transduction cascades and also regulate biological processes such as apoptosis, cell proliferation and differentiation (18). At the molecular level, apoptosis caused by excessive ROS is activated by the aspartate-specific cysteine protease (caspase) cascade, including caspase-12 and -3 (19). Cai *et al.* (20) found that hydrogen treatment significantly suppressed caspase-3 and -12 activities in a neonatal hypoxia-ischemia rat model, which could be conjectured that hydrogen has the property to inhibit apoptosis *via* scavenging the ROS.

Nevertheless, recent research done by Yasunori Sato showed that hydrogen can permeate into mitochondria and directly reduce the production of superoxide (21). As we know, mitochondrial respiration is a major source of ROS, superoxide anion can be generated through a way of electron leak of respiration chain and the reactive oxidant species (ROS) can be formed in the further reaction in the mitochondria. They are also produced by ionizing and UV radiation and from the metabolism of a wide spectrum of drugs and xenobiotics. Beyond that, free radicals arise through the autoxidation catalyzed by transition metal ions (22). Superoxide dismutase converts O_2 into hydrogen peroxide (H_2O_2) which is detoxified into H_2O by either glutathione peroxidase or catalase. Excess oxygen can react with hydrogen peroxide to produce hydroxyl radicals by the Fenton reaction, under the action of transition metal ions such as Fe^{3+} and Cu^{2+}. Because of its low molecular weight, it can easily permeate the membrane including the mitochondrion. So we can assume that hydrogen enters the mitochondrion to influence the respiration chain to inhibit the synthesis of the hydroxyl through interfering with activities of the transition metal which can be treated as a special kind of free radical, reducing the Fenton reaction activities. Also with the other enzymes including NADPH oxidases (NOXs), xanthine oxidase, nitric oxide synthase and peroxisomal constituents generate ROS (22). We also presume that hydrogen can affect the TCA cycle and protein synthesis, as well as allowing ATP

and GSH levels as well as induction of Nrf2 and phase II detoxification proteins to withstand the oxidative stress. The mechanism of induction may result from ROS-mediated dissociation of the Keap1-NRF2 complex. NRF2 then binds to antioxidant response elements and induces the expression of endogenous antioxidant enzymes (23,24).

Hydrogen acts as the fourth gaseous signaling molecule after NO, CO and H_2S

Tomohiro Itoh (8) established a model of immediate-type allergic reaction in mice to check the efficacy of hydrogen. Before this research hydrogen effects had been solely ascribed to exclusive removal of hydroxyl radical, but the immediate-type allergic reaction was not causally associated with oxidative stress. However in this experiment oral intaking of hydrogen-rich water abolishes an immediate-type allergic reaction conspicuously. Experimenters found that hydrogen attenuates phosphorylation of the FcεRI-associated Lyn and its downstream signal transduction, which subsequently inhibits the NADPH oxidase activity and reduces the generation of hydrogen peroxide. They also found that inhibition of NADPH oxidase attenuates phosphorylation of Lyn in mast cells and its downstream targets including Syk, PLCγ1, PLCγ2, Akt, ERK1/2, p38 and cPLA2, indicating that the most upstream FcεRI-associated Lyn is the target of the feed-forward loop and hydrogen accordingly inhibits all tested signaling molecules in the loop. They propose that the hydrogen effect on the signal transduction is that hydrogen compromises the initial step, the phosphorylation of Lyn, which subsequently attenuates molecules within the loop. They propose that hydrogen exerts its beneficial effect by modulating a specific signaling pathway which was not clear enough. The results imply that hydrogen may ameliorate a wide variety of diseases, irrespective of their causal association with oxidative stress, through modulating yet unidentified signaling pathways.

Previous studies have reported on the beneficial effects of gases such as nitric oxide, carbon monoxide and more recently, hydrogen sulfide in animal models of many diseases (25-27). Cardinal *et al.* (11) described the use of hydrogen as a treatment for chronic allograft nephropathy (CAN) in a rat model of kidney transplantation. They

found some interesting results, a sustained increase in the levels of hydrogen in the kidney and serum and improved kidney allograft function over a 60-day follow-up period. Notwithstanding hydrogen antioxidant ability is generally accepted, it is certainly not the sole explanation. Precedent for this possibility can be easily found in other gas-generating systems, just like NO, CO and H_2S, which play a physiological role and participate in several signaling pathways known to modulate inflammation and other pathology procedures (26). In Katherine Wood's comment on Ohsawa's experiment (28) it was shown that there are still some questions; however, whether these concentrations of hydrogen gas could compete effectively with the numerous cellular targets of the hydroxyl radical, membrane lipids and thiols are in far greater abundance than the hydrogen gas molecules successfully used in these experiments. Furthermore, the published rate constant for the reaction of •OH with H_2 to form H_2O and H• is drastically slower than most radical-radical reactions (4.2×10^7 $M^{-1}sec^{-1}$ *vs.* 10^9 $M^{-1}sec^{-1}$) (29). So we can also hypothesize when exogenous hydrogen is emitted to microcirculation, its low concentrations of hydrogen can act as a promoter of protective physiological process in the human body and this promoter mainly conducts the function as a signaling molecule.

Hydrogen and the cancers

The relation between oxidative stress and oncogenesis is very close-knit. Firstly, carcinoma cells are frequently exposed to persistent oxidative stress (30). Shinya reported that human tumors have a content of 8-OH-dG that is ten times as much as the adjacent non-tumorous tissue (31) which is mutation-prone (G:C to T:A transversion) (32,33). Excess reactive oxygen species (ROS) has the property of carcinogenesis by causing strand breaks, alterations in guanine and thymine bases, and sister chromatid exchanges (34). Sub-lethal oxidative stress can also activate the mitogen-activated protein kinases (MAPKs) pathways to promote cell proliferation (extracellular signal related protein kinase; c-Jun amino-terminal kinase/stress-activated protein kinase; and p38 (35).

On the other hand, human tumor produces ROS at the faster speed than normal cell

lines (36); firstly because the NADPH-oxidase is regulated by the GTPase Rac1, which is itself downstream of the proto-oncogene Ras (37). In addition it has something to do with thymidine phosphorylase. It catabolizes thymidine to 2-deoxy-D-ribose-1-phosphate which can generate ROS within the carcinoma cell quickly (38). Thirdly, because tumor rapidly outgrows the blood supply, it will stimulate blood vessel formation (angiogenesis) in a short time. Blood flow within these new vessels is often chaotic, causing periods of hypoxia followed by reperfusion, which can cause the generation of ROS (39). Tumors are frequently infiltrated by large numbers of macrophages and TNF-α is secreted by tumor-associated macrophages, which can induce cellular oxidative stress (40).

ROS increase the angiogenic factors IL-8, vascular endothelial growth factor (VEGF) and matrix metalloproteinase-1 (MMP-1) in the tumors, which can promote vessel growth within the tumour microenvironment (38). Oxidative stress may also increase the blood supply to carcinoma by triggering vasodilatation. It can induce inducible nitric oxide synthase (NOS) (41) and heme oxygenase-1, which can degrade heme to biliverdin and carbon monoxide (38). NOS and carbon monoxide can activate cGMP within nearby smooth muscle cells leading to vasodilatation. ROS can activate MMP-2 (42) and inhibit anti-proteases (43), includingα1-proteinase inhibitor, α2-macroglobulin, plasminogen activator inhibitor and plasmin inhibitor enhancing action of proteases, such as elastase, plasminogen activator and plasmin. These may facilitate tumor invasion and metastasis.

So when Dole *et al.* (2) did the research of hairless albino mice with squamous cell carcinoma exposed to a mixture of 2.5 percent oxygen and 97.5 percent hydrogen at a total pressure of 8 atmospheres for periods up to 2 weeks, marked regression of the tumors was found. Firstly, they showed that hyperbaric hydrogen therapy might have the ability to affect the cancers. The mechanism they proposed was that the hydrogen might firstly act to scavenge the ROS; for example, the hydroxyl was described as the most damaging of free radicals. Another mechanism was that the hyperbaric exposure to a reducing atmosphere interferes with the respiration and metabolism of the cancer cells, functions which are different from those of normal cells. The previously

overwhelmed immune system could then better cope with the squamous cell carcinomas. It is reported that the hydrogen-dissolved water can decrease the ROS, protect DNA, RNA and protein from oxidative damages and it can also suppress tumor-preferential clonal-growth-inhibition, tumor invasion and tumor angiogenesis. Saitoh (44) found that anti-cancer activity of Pt-nc-supplemented HD-water was shown by its preferential cell-growth inhibition to human tongue carcinoma cells HSC-4 over normal human tongue cells DOK and might be partly attributed to HD-water-caused enhancement of Pt-nc-relevant antioxidant ability. Some data shows that the decrease in ROS generation changed biochemical signaling pathways *via* redox-sensitive molecules like AP-1 and NF-κB, interrupting the cascade pathway, which may promote cell transformation (45).

Endogenous hydrogen acts an important role in the human protective system

It is well known that some intestinal bacteria, such as *Escherichia coli*, can produce a remarkable amount of molecular hydrogen (H_2) through the fermentation of the non-digestible carbohydrates in the large intestine (46,47). It was also demonstrated that H_2 concentrations in live mouse stomach or livers (about 20-80uM) are over 20 times greater than the apparent whole-cell Km for hydrogen (48,49). When Mikihito Kajiya (50) measured the molecular hydrogen (H_2) produced in organs of mice by using a needle-type hydrogen sensor, they found that the amount of H_2 detected in the caecum was highest, followed, in descending order, by the small intestine, large intestine, liver, spleen and blood. A trace level of H_2 was detected in the brain. Maier *et al.* also found that the average hydrogen concentration is over 53μmol/L (51). Molecular hydrogen levels ranged from 118 to 239μmol/L in the small intestine of live mice (the mean value for 12 determinations was 168mol/L) and spleen and liver tissue hydrogen levels were approximately 43μmol/L (52,53). The concentration of hydrogen in some mice tissues reached the antioxidant effect in their paper demonstrates; for example the hydrogen in the liver reached almost 60μmol/L, while the effective dose detected while exogenous hydrogen was introduced was only 25μmol/L. However, when the oxidant stress or the inflammation happened, the

endogenous hydrogen did not show the conspicuous effect until the exogenous hydrogen was introduced. Thus, we think that hydrogen should firstly be an endogenous antioxidant in the body and also that hydrogen may act as a signal molecule which can promote the defense of the body.

Accumulated lines of evidence have suggested that intestinal resident bacteria possess a host protective function in the context of their commensal host relationship (54,55). However, the gases produced with fermentations are not utilized by the host but are primarily either lost in faeces or flatus or assimilated by methane producing bacteria (56,57). Some data also showed that almost 14% (58) or 20% (56) of the total colonic hydrogen production was reported to be carried through the human bloodstream and then released into the lung. So the relatively low anti-inflammatory potency of H_2 released from intestinal bacteria compared with exogenous intake have different reasons, most plausibly attributed to the scavenging of H_2 by other bacteria present deep inside the intestinal mucosa or in the stomach, such as *Helicobacter hepaticus* which is reported to consume significant amounts of H_2 (52). Since most mammalians lack the catabolic enzyme to generate H_2, intestinal bacteria are the only possible source of protective H_2 (56,59). Thus, exogenous factors, such as systemic antibiotic treatment may alter the number of host protective commensal bacterial flora in the intestines, ultimately resulting in affecting the functional amount of H_2 and consequently, the organism's susceptibility to disease. Some food and medicine can promote the generation of the endogenous hydrogen, such as raffinose, lactose, fructose, sorbitol, starches, soluble fiber, insoluble fiber, dietary turmeric, manicol, acarbose and so on.

The merits and demerits of hydrogen therapy

To detect the potential demerits of hydrogen therapy, Saitoh *et al.* (44) observed the mutagenicity, genotoxicity *in vivo* and subchronic oral toxicity in a rat model and found a few statistically significant changes in hematology and clinical chemistry parameters. In humans, Nakao *et al.* (60) discovered decreases in aspartate aminotransferase and alanine aminotransferase and increases in gamma-glutamyl

transferase and total bilirubin, but all of these changes remained within an acceptable clinical range. Moreover, loose stools, increase in frequency of bowel movement, heart diseases and headache were observed in the experiment which were considered to have a relationship with hydrogen therapy (61). However there are still a lot of advantages over some pharmaceutical drugs. For instance, in spite of few toxic effects at the effective concentrations of hydrogen reported, overdosing is impossible because excess hydrogen is excreted through the respiratory movement of the lung. Another advantage is the property of selectively scavenging the most aggressive ROS, •OH, but has little impact on other ROS like O_2-• and H_2O_2 which plays an important role in the normal physiological activities. Moreover H_2 does not influence physiological parameters such as temperature, blood pressure, pH or pO_2, which are very important for homeostasis. Thirdly, because of its low molecular weight, it rapidly diffuses into tissues and traverses the membrane including the mitochondria and the nucleus to reach important targets. Finally, hydrogen has multiple functions such as antioxidant effects, anti-inflammatory effects, inhibition of apoptosis, anti-allergic effects, anti-cancer effects, *etc.*, so the treatment spectrum is broader than other areas of medicine.

Prospects of hydrogen research

Although much work has been done in the last few years, hydrogen medicine is still a hotspot since there are still so many unresolved problems. Firstly, hydrogen therapy still remains to be proven effective against many diseases. Moreover, at the molecular level, it is unclear how H_2 achieves site of action and selectivity combines the first target while there are thousands of possible target molecules. Of course, the exact first target molecule is the key to the mysterious hydrogen kingdom. The pathways and processes regulated *in vivo* by hydrogen need to be detected. On the basis of previous work, we also need to do a lot of research to formulate a standard which can provide the optimum dose, timing and delivery methods. In addition, in spite of few negative effects reported, the disadvantages and toxicity of hydrogen gas should be researched further before clinical application.

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TABLE 1. Recent researches of hydrogen *via* various disease models.

Organs or systems	Animal models	Reference
Central nervous system	1.Focal cerebral I/R injury model	Ohsawa *et al*. (1)
	2.Hypoxia-ischemia insult occurs in the perinatal period	Cai *et al*. (14,20)
	3.1. Models of moderate and severe neonatal brain hypoxia.	Matchett *et al*. (62)
	3.2.model of middle cerebral artery occlusion in an adult rat focal ischemia	
	4. Stress-induced decline in learning and memory	Nagata *et al.* (63)
	5. Hypoxia-reoxygenation model of the brain	Sato *et al*. (21)
	6. Parkinson's disease	Fujitaet *et al* (64); Fu *et al*. (65)
	7.Alzheimer's-like rat model	Li *et al*. (66)
	8. Model of acute spinal cord contusion injury	Chen *et al*. (67)
Cardiovascular system	9. I/R injury induced by transient occlusion of the coronary artery in rats	Hayashida *et al*. (68)
	10. Heart transplantation	Nakao *et al*. (60)
	11. Atherosclerosis	Ohsawa *et al*. (69)
Lung	12.ventilator-induced lung injury and hyperoxia for 60h inducing lung edema	Huang *et al*. (70)
	13. Lung injury induced by intestinal I/R injury in a rat model	Mao *et al*. (6)
	14. Lung transplantation	Tomohiro *et al.* (71)
	15. COPD	Shu *et al.* (72)
	16.pulmonary hypertension	Yun *et al.* (73)
Renal system	17.Cisplatin-induced nephrotoxicity	Naomi Nakashima-Kamimura *et al*. (12)
	18.Renal transplantations late failures attributable to chronic allograft nephropathy	Cardinal *et al*. (11)
Liver	19.Liver warm I/R injury in a mouse model	Fukuda *et al*. (36)
	20. Concanavalin A (Con A) induced hepatitis	Kajiya *et al*. (50)

	21.Schistosomiasis-associated chronic liver injury	Gharib *et al*. (74)
	22. Obstruction jaundice with ligation of the bile duct	Liu *et al*. (75)
	23. Liver Injury by CCL4	Han *et al*. (76)
Pancreas	24.Severe acute pancreatitis	Chen *et al*. (77)
Intestine	25.Small intestinal transplantation	Buchholz *et al*. (78)
	26. Model in rats Dextran sodium sulphate DSS -induced rodent colitis	Kajiyama *et al*. (4)
	27. Intestinal contractile dysfunction and damage induced by intestinal warm I/R injury	Chen *et al*. (79)
	28. irritable bowel syndrome	Pimentel M. (80)
Eyes	29. Transient elevation of intraocular pressure	Oharazawa H (81)
Auditory system	301.Cochlear hair cells in vivo by generating ototoxicity	Kikkawa *et al*. (82)
Allergic reactions	31An immediate-type allergic reaction model	Itoh *et al*. (8)
Metabolism	32.type-2 diabetes	Kajiyama *et al*. (3)
	33.potential metabolic syndr -ome	Nakao A *et al*. (83)
	34. patients who were treated with acarbose	Suzuki *et al.* (9)
Infection	35. polymicrobial sepsis and sepsis-associated organ damage in mice	Xie *et al.* (4)
Tumorigenesis	36. skin tumours in hairless albino mice with squamous cell carcinoma.	Dole *et al*. (2)
	37. The culture of human tongue carcinoma cells	Saitoh *et al.* (44)
Radio protective effects	38.ionizing irradiation-induced human lymphocyte (AHH-1) cell apoptosis and increase cell viability in vitro	Qian *et al.* (84)
	39. Acute radiation syndrome	Liu *et al.* (85)
	40.Radiation induced Thymic Lymphoma	Zhao *et al.* (86)