Investigation of Isoflurane in „in vivo” animal experiments

Phd Thesis

Balázs Kádár MD

Head of School: Prof. Sámuel Komoly MD PhD DSC
Program leader: Prof. István Ember MD PhD DSC

Institute of Public Health
Faculty of Medicine
University of Pécs

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Introduction

Inhalation anesthetics are used from decades in anesthetic surgical procedures. During the last decades anesthetically instruments have been upgraded significantly and inhalation narcotics became much safer to use. However, the exposure and possible health damage of the staff working in operation theatre, remains object of study until recent times. Our study focuses on the gene expression with possible effect of carcinogenesis and apoptosis of this very common anesthetic agent the Isoflurane. For our experiments we used animal models. We tried to prove that constant use of anesthetic gases in operating theatre can cause individual complaints and chronic diseases. The aim of our investigation is to increase workplace prevention, safety and to decrease exposure.
Observing the metabolism and toxicology of Isoflurane, we can understand supposed gene expression and molecular changes, and the possible connection of them with carcinogenesis.

Properties of Isoflurane, metabolism and toxicity

The Isoflurane itself is a non flammable liquid, can be dosed by vaporisator, a common anesthetic narcotic. 1 cloro-2,2,2 trifluroetildiflurometil ether. Isoflurane is a clean non colored stabile liquid do not contain chemical stabilizers or any other additives. It has a pungent odor.
The molecular structure of Isoflurane is very stabile, thanks to three fluor atoms present on the terminal ethyl carbon atom. It is hard to enter in chemical and physical reactions, does not react with metal and it is stabile in soda lime and ultraviolet light. In comparison with the Halothane base molecule the Isoflurane has the chlorine atom exchanged with fluor atom resulting in the reduction of blood/gas partition coefficient. As a result we have a rapid anesthesia and a quick elimination. The uncommon nephrotoxicity and hepatotoxicity is a result of the acetylation protein, caused by trifluoroacetic metabolite. Isoflurane passes through the microsomal defluorination, catalyzed by the P450 2E1 cytochrome enzyme. Released ionic and non ionic products are responsible for DNA damage and other biological effects of lipid peroxidation, antioxidant depletion and formation of adducts.
Metabolization of Isoflurane is relatively low in the human organism.
A low level of 0,17% can be found in form of metabolite in the urine during the post operative period.
Thanks to a minimal bio-transformation and low blood/gas coefficient of Isoflurane, the amount held back of the substance in adipose tissue after anesthesia is transformed in intermediary and toxic metabolites.
Metabolism of Isoflurane is catalyzed by the CYP2E1. The process produces a collateral product trifluoride-acyl ester difluorometil; this product enters in reaction with water and decomposes in trifluoride acetic acid and non organic fluorides.
The trifluoro-acil-difluoro-metil ester and the trifluoroacetate are capable through the CF_3CO metabolite hapten to do a covalent binding with the proteins containing lysine of the liver. This process produces an altered protein, causing an immune reaction. This mechanism occurs in the formation of hepatitis. Production and selection of the metabolites is done by the lung and kidneys. The general peak concentration of the non organic fluoride of the serum is less than 5 μmol/l. Peak values are present generally after 4 hours of the anesthesia, after 24 hours we can find normal values. More less 50 μM/L is a necessary level of fluoride to cause an acute nephrotoxicity.

Aspects of molecular epidemiology

Molecular epidemiology is looking forward to understand connections especially early connections between exposure and clinically manifesting diseases using tools of the modern molecular biology.
Standard workplace safety procedures and their legal background in connection with clinical exposure to clinical anesthetic gases are deficient in Hungary. It is very important to monitor potential biological effects of the agent of exposure, using molecular epidemiological markers, in order to have an early feedback. In order to produce a standard working workplace and staff protection procedure, we need to run all necessary animal experiments and find the early biological markers. It is obvious in the case of inhalation anesthetics especially when the organism is in stressed situations, to examine onco suppressor key genes.
The method can be used to evaluate appropriate both genetic factors (physical, chemical, biological) and individual factors of sensibility of the organism (genetic, epigenetic immunological, nutritional). In consequence we can evaluate mechanism of action of some infectious and chronic non-infectious diseases.
Existent data in literature about Isoflurane, focuses on ambient concentrations and present of metabolites in the organism, describing internal dose of the compound. Examinations about genotoxicity of the compound (examination of chromosome aberrations comet-assay, use of sister chromatid exchange) describe the biologically effective dose and early biological impacts of Isoflurane.
In our experiments we will demonstrate possible changes in expression of onco/suppressor key genes and also changes in function of the inflammatory signal transmission systems. We analyze connections between the early biological effects of Isoflurane and manifestation of disease (inflammations, dysplasia). Our results make possible, revealing feedback reactions of early biological markers, a preventional, precise and standard biological monitoring system for occupational health purposes.

Objectives

Many examinations of inhaled anesthetics attempted to clarify organotoxicity of the anesthetics. It is difficult, however to establish long-term health consequences with such regular and long-term exposure and below threshold dose, in the vast majority of cases there is no obvious organotoxic effect.
During our investigations using methods of molecular epidemiology, we studied onco and suppressor genes commonly activated in human tissues, and in inflammatory processes, specially the expression of key genes. These genes can be detected at a below threshold exposure and interpreted as potential biomarkers in the process of multistage carcinogenesis. Our examinations of the gene expression is based on constant use of inhalation anesthetics, specially of the exposed population, primarily of staff working in operating theater and chronic patients operated several times, risks of tumor development and to identify individuals with increased risk factors. The analysis of the tissue specific gene expression patterns give a real possibility to describe the most exposed organs.
Our experiments had been conducted using Isoflurane a very commonly used inhalation anesthetic agent which has the lowest organotoxical character.
Using our experimental method we can detect the significant risk, caused by high or below threshold but chronical exposure. Using tools of the primary prevention such as increased controls and use of work hygiene regulations we can efficiently decrease and prevent the health damage caused by high exposure to Isoflurane or any other inhalation anesthetics.

Study plan

1., We planned to evaluate effects on gene expression „in vivo" in animal experiments of Isoflurane, regarding the expression of genes *p53, c-myc, Ha-ras.* We used H-2^k haplotype, CBA/Ca mice, sensibilized to chemical carcinogens.
2., We tested the effects of Isoflurane in the expression of *GADD45α,* an indicator of single-stranded DNA damage, *NFkB,* which has a major rule in the regulation of apoptosis and *MAPK8* genes in samples taken from the organs of CBA/Ca H-2^k mice.
3., We conducted a human questionnaire survey to collect statistical data, regarding possible health status consequences of exposure to inhalation anesthetic gases, formation of possible somatic and neuro-endocrine diseases, occurrence of cognitive changes.

In our study we try to demonstrate that below threshold dose of Isoflurane in chronic exposure may be damaging health conditions of employees working in operating theater and this fact can be monitored. It is crucial to raise awareness about the treatment of anesthetic gases, work safety deficiencies in legislation and procedures, propose technological improvements, increase control and solidify work safety procedures.
The most important aspect of the present thesis is the primary prevention using methods of molecular epidemiology, description of the importance and methodology of bio-monitoring.

Materials and methods

Planed experimental animal model for the characterization of genomic effects of Isoflurane

In our experiments we used five weeks old CBA/Ca H-2^k haplotype mice sensibilised to chemical carcinogens. The average weight of the animals where about 20-23,5g. The CBA/Ca mice substrain is a commonly used experimental animal for the test of the formation of tumors in mammals. These mice are in the histocompatibility substrain as H-1^a, H-2^k and H-3. The CBA/Ca mice are commonly used in the research of leukemia, since they are sensible to chemical agents, and they present a low level of spontaneously manifesting leukemia.
Our experiments have been conducted in the University of Pécs, Institute of Public Health Medicine, in laboratory conditions, at normal room temperature, at an average humidity level, at 1014 hPa atmospheric pressure.
In our „short-term" animal experiments we focused on the effects of Isuflurane on onco and tumorsuppressor genes, like *c-myc, Ha-ras, p53, GADD45α* as an indicator of single-stranded DNA damage, *NFkB* having a major rule in the apoptosis, and the *MAPK8* genes.
After a 1 hour long exposure to Isoflurane, inhaled at the concentration of 2 percent, the test animals were divided in groups containing 6 mice, the autopsy where conducted in 3 specific time. Examined organs where extracted after 3, 24 and 48 hours of the termination of anesthesia. Samples where isolated and tested separately from the body of male and female animals. We tested samples from seven preselected organs, from the liver, lung, spleen, kidney, bone marrow, lymph nodes, thymus, looking for the exposure of the onco genes *c-myc* and *Ha-ras*, and in the suppressor gene *p53*. We conducted a numerical and a bar graph analysis. In the control group we tested gene expression after 1 hour of exposure at 100% oxygen, of 6-6 male and female animals.

In the Isoflurane group measured gene expression changes where expressed in β aktin percent, the results were divided with the results of the control group, resulting in a difference ratio between the two groups. For the calculation of significance we used a two taled two sample equivalent variance t test.
During the following test we separated 6 different groups. In the case of three groups we inducted a 1 hour long Isoflurane narcosis, and for three groups we doubled the exposure time to two hours. Concentration of Isoflurane during this test was also calibrated at 2%. In the case of both genders we performed the autopsy after 6 hours from the end of the exposure for the first group, and 12 hours after the end of the anesthesia for the second group. Animals belonging to the control group, were exposed to 2l/min oxygen flow for 1 and 2 hours (1-1 group per gender).
During autopsy lung, liver and kidney where removed. We isolated high purity total RNA from tissue samples of the extracted organs. High purity total RNA was used in quantitative real-time PCR through Light Cycler instrument. We used an amplification kit to determine absolute mRNA content of the tissues for *GADD45α*, *NFkB1, MAPK8* and HPRT in relation to the relevant absolute nucleic acid content of mRNS. All PCR reactions where done in three technical replicates, in three different running cycles. We averaged the obtained results by tissues and by genes as well.

Studies based on human questionnaires

Taking under observation the Hungarian reality of the workplaces and operation theatres, as well as the standards methods of anesthesia we can observe a variety of differences. In our experiment we collected questionnaires from staff working in anesthesia in different institutions in order to comprehend all the possible subjective signs and also examine test results of the staff, looking for possible signs and symptoms of continuous exposure to anesthetic gases used in operation theatres. We focused on the possible differences in work-safety checkups of employees and their regularity in different institutions. Questionnaires have been taken from multiple institutions, from the Department of Surgery I. of the Semmelweis Medical University, Budapest, from the Department of Neurosurgery and Pediatric Surgery of the Medical University of Pécs, from the County Hospital of Baranya, from the Hospital Józsa András of Nyiregyháza, from the Central Department of Anesthesiology and Intensive Care of the Teaching County Hospital of Borsod-Abaúj-Zemplén, from the Central Department of Anesthesiology and Intensive Care of the Erzsébet Hospital, Sátoraljaújhely, and from the Hospital of the City of Kazincbarcika.
For the evaluation of the questionnaires we used MS Office Excel 2007 and IBM SpSS Statistics.

Results

Results of test of gene expression of mRNA

Graphs made from the analysis of the exposure to Isoflurane and oxygen where demonstrated alongside each other for a better comprehension. Females and males are shown simultaneously. Figures shown in the dissertation are containing four independent graphs. (Figure 1-2) We compare the onco suppressor gene expressions isolated from the seven most

exposed organs like liver, spleen, lung, kidney, thymus, lymph nodes, bone marrow, which either are involved in the metabolism of the anesthetic gas or have a relevant function.
Examining the organotoxicity of inhalation anesthetics, the involvement of the liver is a proved fact; consequently Isoflurane can cause liver damage even at the point to cause hepatitis. Involvement of the liver is clearly and significantly represented in our examinations. *(Figure 1.)*

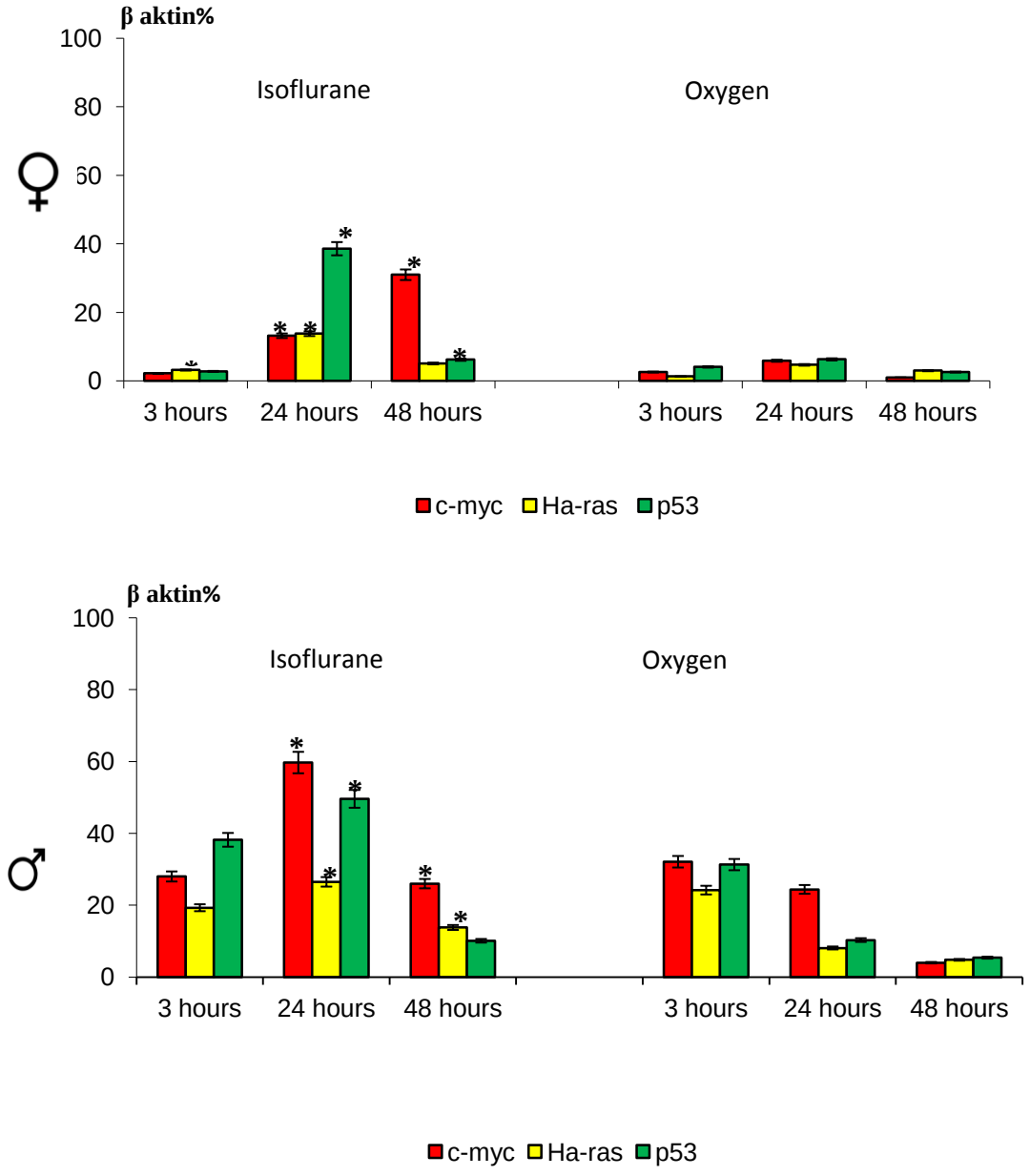

Figure 1.
c-myc, Ha-ras and p53 gene expression, in percent of β-actin level, after 1 hour of exposure to Isoflurane, and 1 hour 100% oxygen inhalation, in the liver of CBA/Ca mice

As we supposed in the planning of the present investigation, we found significant and major discrepancy in relation to the over expression of the onco suppressor genes in lung, which is the most exposed organ to inhaled anesthetic gases. *(Figure 2.)*

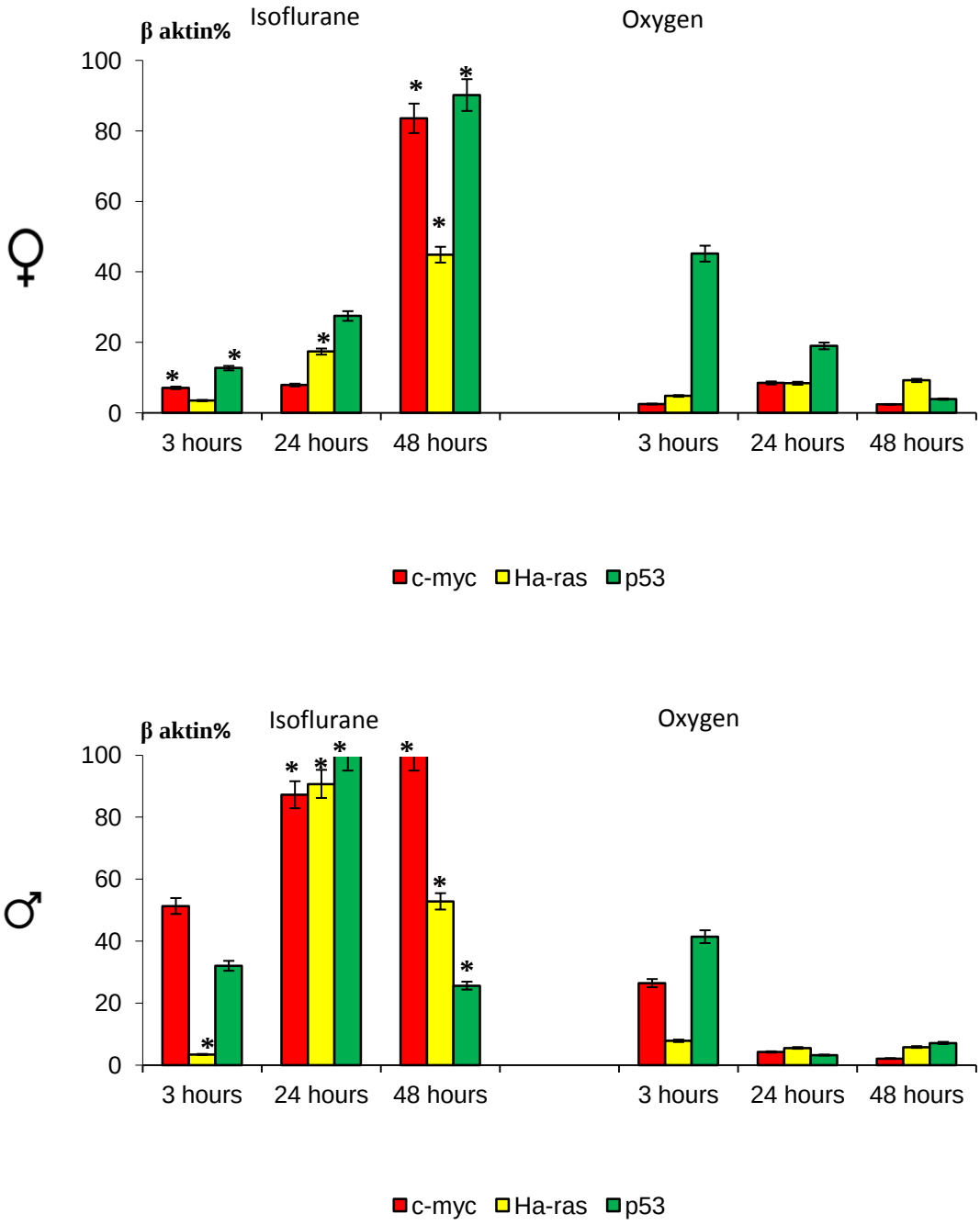

Figure 2.
c-myc, Ha-ras and p53 gene expression, in percent of β- actin level, after 1 hour of exposure to Isoflurane, and 1 hour 100% oxygen inhalation, in the lung of CBA/Ca mice

Test results of gene expression of the inflammatory markers

Data obtained after a period of 1 and 2 hours of exposure to Isoflurane has been compared with data coming from the control group. We did examine singular genes one by one and also the gene expressions of genes *NFkB, GADD45α* and *MAPK8* between each other.
After an exposure to Isoflurane for a period of six hours we noticed in lung and renal tissue a significant change in all three gene expressions in comparison with the control group. In the case of *NFkB* after 2 hours, while in the case of *GADD45α* and *MAPK8* 1 hour of exposure was enough to obtain significant results in samples taken from lung tissue. After six hours of exposure to Isoflurane, the expression of *NFkB* in lung increased to reach double level, compared to the results of the control group in the groups exposed for 2 hours (times 2,09, p=0,0025). Expression of *GADD45α* and *MAPK8* compared to the control group resulted in a significant decrease in the group exposed for 1 hour to Isoflurane. In the case of the *GADD45α* after 12 hours the difference was times 0,27, p=0,0053, while in the case of *MAPK8* gene after 6 hours times 0,42, p=0,027, after 12 hours times 0,49, p=0,035. On the other hand we can observe the exact opposite results in kidney tissue. After 6 and 12 hours the expression of *NFkB* was decreased, compared to the control group, and this reduction was significant in tissue samples taken from the two hours exposed groups in both autopsies conducted in different moments. Over expression of *GADD45α* can be observed in the kidney after 1 hour of exposure to Isoflurane, as well as the expression of *MAPK8* increased significantly, in the case of those animals whose autopsy was conducted 6 hours later, counting from the end of the 1 hour exposure (times 3,47, p=0,015). The expression of the gene *GADD45α* in kidney after 1 hour of exposure, but removed 12 hours later was expressly high, without reaching the level of significance.
In our experiments the expression of *GADD45α* and *MAPK8* were resulted often parallels, while *NFkB* several times shows the opposite gene expression results.

The gene expression of *NFkB* which regulates the cell survival by the inhibition of the apoptosis, in the samples taken from the lung - the most exposed organ to Isoflurane - after 6 hours from the end of the narcosis was increesed in both groups compared to the results of the control. After two hours of exposure, levels were elevated to more than twice, which was significant. (p=0,0025). *(Figure 3.)*

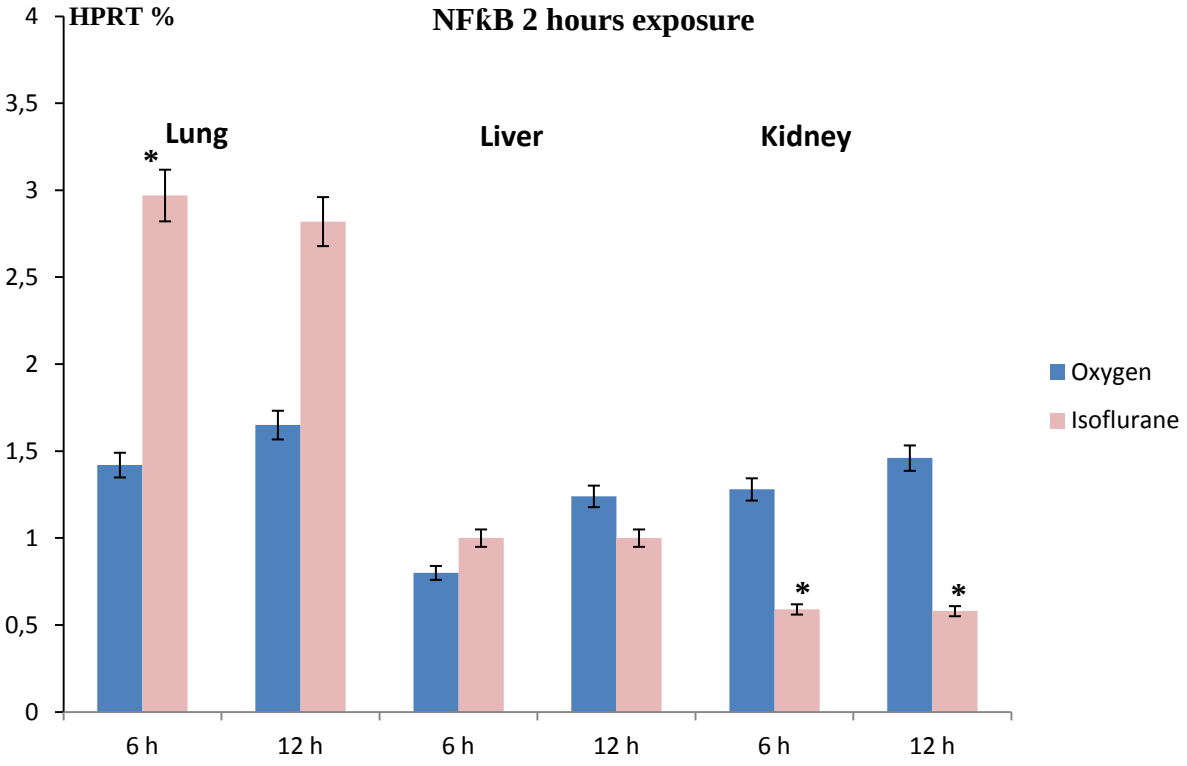

Figure 3.
NFkB expression in Hprt ratio, after 2 hours of exposure to Isoflurane 2%, and after 2 hours of inhalation of oxygen 100%, from sample taken in autopsy conducted in the interval of 6 and 12 hours, from liver, lung and kidneys of CBA/Ca mice

Examination of the *GADD45α* gene - which is activated by the damage of the single-stranded DNA – resulted in opposite changes from the experienced in case of the antiapoptotical gene *NFkB*. *(Figure 4.)*

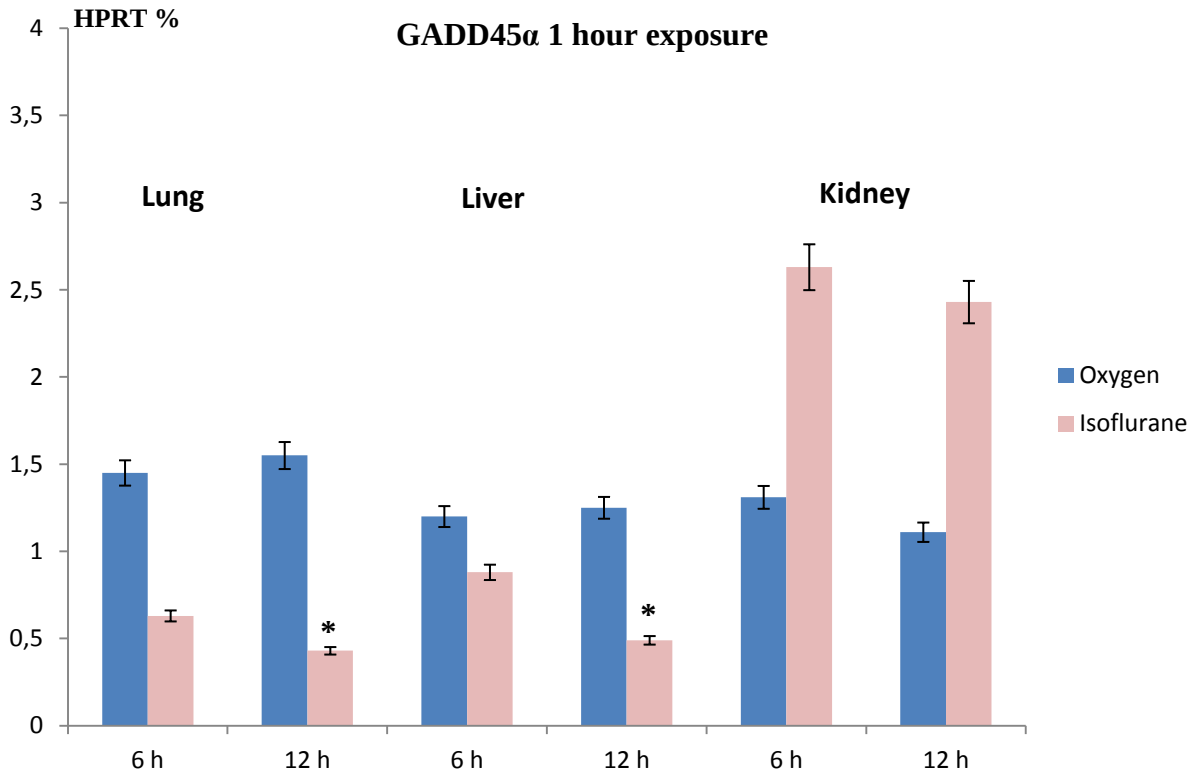

Figure 4.
GADD45 expression in Hprt ratio, after 1 hour of exposure to Isoflurane 2%, and after 1 hour of inhalation of oxygen 100%, from sample taken in autopsy conducted in the interval of 6 and 12 hours, from liver, lung and kidneys of CBA/Ca mice

Gene expression pattern of the *MAPK8* - which indicates apoptosis and is in tight signal transmission with *GADD45α* – is in several points similar to the results produced by *GADD45α. (Figure 5.)*

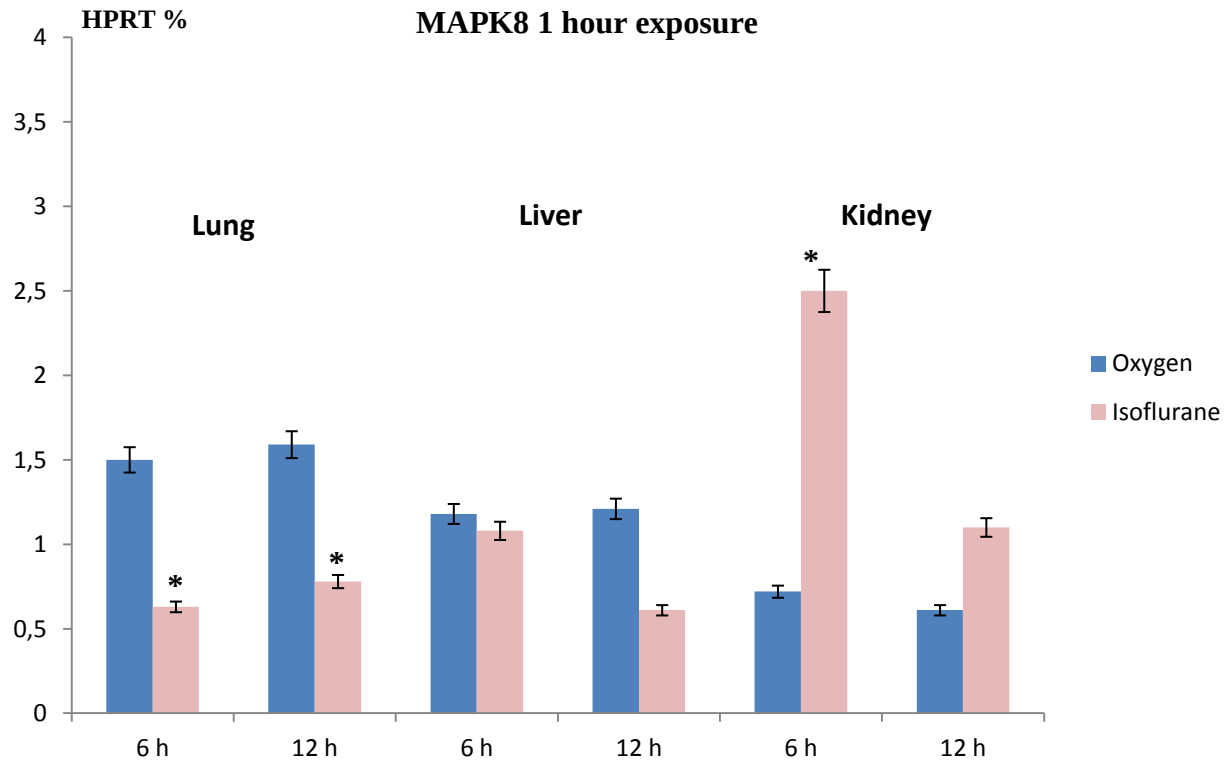

Figure 5.
MAPK8 expression in Hprt ratio, after 1 hour of exposure to Isoflurane 2%, and after 1 hour of inhalation of oxygen 100%, from sample taken in autopsy conducted in the interval of 6 and 12 hours, from liver, lung and kidneys of CBA/Ca mice

Statistical evaluation of human test questionnaires

After collecting the completed questionnaires, we evaluated 104 questionnaires. From the test subjects 27 were men, 75 were women 2 people did not specify any gender. Average age were 41 years. Regarding their positions 59 of them were doctors or residents, 44 of them were anesthetic assistants.
From the human test pool, 31 people (29.8%) works in this current role from less than 5 years, 37 (35,5%) works in anesthetic role from more than 20 years.
One person gave the answer of working in average less than 1 hour a day in operating theater. Six people indicated (5,76%) a time frame of 1 to 4 hours, and 52 people answered to work 4 to 8 hours in operating theater. 37 of them (35,57%) works from 8 to 12 hours and six people (5,76%) answered to work even more than 12 hours a day in operating theater close to an anesthetic machine. In the process of creation of the questionnaires we focused on the following problems. Connections between some subjective complaints and exposure time in operating theater to anesthetic gases and number and ratio of inhalation anesthesia, measured distance from anesthesia apparatus, measured distance from floor level. Generally we tried to understand how these factors alter health status and possible laboratory abnormalities.

Discussion

Cells react to environmental impact with series of intracellular molecular reactions, from one side to maintain unchanged their physiological functions, from the other side to compensate all functional structural damages caused by the environmental conditions. Molecular epidemiological research and investigations of the environmental exposure, carcinogenic agents and other stress factors are in place from decades at the Institute of Public Health of the Faculty of General Medicine of the University of Pécs. An in vivo animal experiment has been developed to examine to evaluate potential carcinogenic effects and factors of our environment, workplace, and nutritional habits. With the use of the „short-term" test system became possible to detect changes of gene expressions some hours after the exposure. The onco suppressor genes *c-myc*, *Ha-ras* and *p53* selected by us and sensible changes of gene expressions of apoptotic and inflammatory markers proved in our examination, that the early biological markers are able to monitor the environmental exposure influencing carcinogenesis and demonstrate changes of cellular functions.
In order to evaluate and comprehend our results in a proper way, we have to understand all these complex regular mechanism, cascades and signal transmission systems, which are influenced by those proteins which are coded by the genes we examined. Over-expression of genes usually generate intensification of protein synthesis, other times they generate apoptosis or on the contrary they have anti-apoptotic influence.

In our experiments we examined the changes of the expression of *c-myc* and *Ha-ras* key genes, the *p53* suppressor gene, the *NFkB* which regulates cell survival by inhibition of the apoptosis, *GADD45α* activated as a consequence of single stranded DNA damage and the *MAPK8* which is in a tight signal transmission conjunction with *GADD45α* and inducts apoptosis, after exposure to Isoflurane in CBA/Ca mice. After inhalation anesthetics exposure, so particular and extensive study of the molecular aspects of genes coding proteins in connection with carcinogenesis has not been done yet.
In females we can find early manifestations of over-expression in spleen and bone marrow. After examinations increased gene expressions were present in both genders after 24 hours of Isoflurane exposure in almost every examined organ. In the same examination point we can observe the over-expression of the onco gene *Ha-ras* in few organs in both genders. Our experiments conducted to results expected by us, regarding the physiological functions of the exposed organs, and the metabolism of Isoflurane, knowing the organotoxic effect of the anesthetic gas. We found over-expressions of *c-myc* and *p53* genes after 3 hours of the exposure in the spleen, kidney, thymus, lymph node, bone marrow of male animals. Influences in the liver were present in both genders. In the other organs we proved that the induction of *Ha-ras* begins later than in the case of the other two examined genes.
The lung, which is the main target organ in the „crossfire" of the inhaled anesthetics, shows a similar gene expression pattern like in the liver and in the spleen. We could deduct similar conclusions after the examination of the thymus, bone marrow and para-aortic lymph nodes. The key role in the immune system and in some aspect similar histological properties can provide explanation to the parallel dissimilarities in our results. The noticeable difference between the two genders could arise from the neurohormonal difference, which can affect the regulation of DNA repair activity and genes taking part in signal transmission.

In the result of our planed experiment to test inflammatory markers, after 1 hour exposure in kidney tissue of CBA/Ca mice we found an increased expression of *GADD45α* which gene signals damage of single-stranded DNA and the paralelly activating *MAPK8* which stimulates the apoptosis of the cells containing damaged DNA. At the same time the *NFkB* - which

stimulates cell survival and has an antiapoptatic effect – showed a decreased expression. In contraposition in the lung after 120 minute of Isoflurane narcosis we could observe that the expression of *NFκB* gene had been increased and the expression of *GADD45α* and *MAPK8* genes have been decreased. As a conclusion of our experiment we can deduct, that the exposure to Isoflurane influences in a significant degree the genes activating as a consequence of DNA damage and also the genes regulation of apoptosis.

Our survey based on human questionnaires was unable to monitor the carcinogen probability of the staff working in operation theater, but the manifestation of subjective complaints correlated in a significant way to exposure to Isoflurane. There were some subjective complaints by the part of the interviewed staff; these cannot be accepted as a consequence of inhalation anesthetics. There were also some significant variations such as bronchial irritation and mucous membrane irritation. Chronic irritation and possible erosion of the bronchial system and the mucous membrane are in the case of other agents proved origin of malignant degeneration. In the case of Isoflurane - which is an irritant substance - we also need to take in consideration such side effects after a durable exposure to the substance. After the evaluation of the questionnaires, we can see an emerging need to monitor and screen the "invisible" deviations in time, with standard methods, with preventive approach which has to be amplified and introduced.

Proposals to increase workplace safety in operating theater and biological monitoring

Our task with the present investigation was amongst other to discover possible health damaging consequences of the use of inhalation gases and especially the commonly used Isoflurane. We tried to prove such consequences and discover early biological markers that can be monitored in a standard way.
We seen from the results of the tests, which halogenized anesthetics can cause physical and psychological tiredness, headache, respiratory tract and mucosal membrane irritation. Use of such gases has negative consequences on cognitive memory. In relation to the above mentioned complaints we can observe a strict correlation between the time spent in operating room and the frequency of inhalation anesthesias, the number of active years of service. Not every employer obligates workers to a half-yearly occupational health screening, as a result of our human questionnaire based survey, we see that with regular screenings, occurrence of laboratory alterations can be decreased by 50%.
Standard physical examinations and laboratory tests of occupational health department are insufficient to indicate adverse health effects of increased exposure to anesthetic agents.
However, presence of inorganic fluoride level in urine can be a sensitive parameter.
The biologically tolerable value of fluoride is accepted from 4.0 to 7.0 mg F^- per gram of creatinine.
The measurement of inorganic fluoride is made with ion-selective electrode; the results can be read after 2-3 minutes, finally followed by use of a calibration curve, expressed in F/g creatinine in urine.
In Hungary, the National Institute of Labour Hygiene and Occupational Health is able to carry out such investigation. In case of a positive finding well above threshold, the examination has to be done again, after a reduced exposure to anesthetic gases.
In case of repeated positive findings we recommend a test of chromosomal abnormalities, mutational analysis and sister chromatid exchange. If genotoxicity is possible, we recommend to use PCR technology in the examination of onco/suppressor genes, as well as the

examination of inflammatory and apoptotic genes. This examination can be conducted relatively quickly in molecular biology laboratories, from leukocytes and lymphocytes isolated from native blood samples, or from macrophages derived from bronchial secretion. It would be desirable to set up and develop such laboratories also from the point of view of public health and molecular epidemiology.
Examination of early biological markers is determinant to evaluate changes of key gene expression in the workplace environment, in our case exposure in operating room, taking into account epigenetic factors as well. The genes *c-myc, p53, Ha-ras, NFkB, GADD45α* and *MAPK8*A are examined by us in animal experiments, they have a relevant role not just in animals but also in human carcinogenesis. These genes can be tested with a single blood sample; the molecular epidemiological test can become a risk estimation process, in which the possible relationship between early effects of carcinogenic genes and the formation of consequent tumors not just can be proved, but also prevented, minimizing occupational exposure. After accurate testing chemoprevention and immunoprophylaxis can be considered in tumor prevention.

Summary and new findings

1., We have several options to measure exposure to inhalation anesthetics of staff working in operating room. These methods are well known but less commonly used in Hungary until recent times. Possible causes of this situation are the insufficient legal background, employers and employees are not sufficiently careful, health and environmental protection is often overlooked.
2., Models describing organotoxicity of Isoflurane – the most commonly used inhalation anesthetic – can be used, in case of an exposure, that significantly surpass threshold levels. Our experiments describes a biological monitoring possibility - providing lasting, sub threshold doses, even for the shortest period of time – that can signal reported health effects of damaging agents, at the most sensitive level of apoptotic, anti-apoptotic, onco and tumor suppressor genes.
3., We conducted two technically different, but under the epidemiological aspects equally important experiments, we first demonstrated, that gene expression of genes playing a significant role in formation of tumors and in the apoptosis, are changed in several tested organs, as an effect of Isoflurane. This means that chronic and even sub-threshold exposure to Isoflurane is an increased occupational health risk for professionals involved in operating theater.
4., We evaluated - using a questionnaire-based method - working conditions of staff working in operating theater their activities associated with the formation of subjective symptoms and observed differences in their observed laboratory values. Significant differences were observed among the people who came into contact with anesthetic gases on a daily basis. Significant differences were attributable to the effect of the anesthetic gas such as fatigue, drowsiness, slow or fast heart rate, mucosal membrane irritation, restlessness. In case of the staff working more than 8 hours a day close to an anesthesia machine the ratio of the development of a number of claims has increased significantly, although it was not statistically significant. Such symptoms are headache, fatigue, drowsiness, and shortness of breath, restlessness, tremor, and dizziness, fear of death, mucosal membrane irritation, and feeling faint.
5., In our experiments we demonstrated the increased expression of *c-myc*, *Ha-ras* onco genes, and *p53* tumor suppressor gene, in several tested organs, after an exposure to Isoflurane.

6., Expression of the genes *NFkB,* - involved in inhibition of apoptosis - *GADD45α* – activated when single-stranded DNA damage occurs - and *MAPK8,* - which inducts apoptosis – in samples from the lung, liver and kidney, is significantly changed as a consequence of exposure to the tested inhalation anesthetic. This demonstrates how exposure to Isoflurane is significantly affecting the activation of genes, which plays an important role in the regulation of apoptosis as a consequence of DNA damage.
7., After our experiments and observing their results, we need to raise awareness about the increased exposure to anesthetic gases and other health damaging consequences of staff working in operating theater. Reduction exposure with technological and workflow policy changes is possible and necessary.
8., For the biological monitoring of staff working in anesthesia we recommend as a first step to measure the inorganic fluoride content in the urine. If test results are exceeding threshold levels, we recommend running the tests again, after the discontinuation of the exposure of the employee to the agent. Subsequently, it is necessary to demonstrate chromosomal differences, to do a mutational analysis, sister chromatida exchange test. We need to test for genotoxic differences. We have to run tests based on PCR technique, about the expression of onco / suppressor, inflammatory, apoptotic and antiapoptotic genes.

Acknowledgments

In 2005 after obtaining my first qualifying specialization, I opted for the anesthesiology as a field of research for my scientific work. I did focus my attention to the Isoflurane, this commonly used inhalation anesthetic gas. I had been working, and I keep working with it also today, day by day. To the elaboration of my scientific method and to perform animal experiments and tests I needed help and support.
I would like to thank first of all Professor István Ember MD, my project leader and supervisor, for his help and support, from the first moments of my work to the last. He gave me ground and place to plan, conduct and evaluate results of my scientific work, in the Institute of Public Health, leaded by him.
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