# A TOLERANCE TO PRESSURE CHANGES DURING EXPERIMENTAL HYPERBARIC OXYGEN THERAPY IN RABBITS

## *TOLERANCE KE ZMĚNÁM TLAKU BĚHEM EXPERIMENTÁLNÍ HYPERBARICKÉ KYSLÍKOVÉ TERAPIE U KRÁLÍKŮ*

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**ABSTRACT**

*Background:* Our paper reports possible complications with hyperbaric oxygen therapy (HBOT) in rabbits. The main concern is a middle ear barotrauma, as it is the most common complication in human treatment. Animal studies usually focus on desired goals, but rarely describe methodological technical issues in detail.

*Methods:* In order to assess rabbit tolerance to compression, we combined several independent methods. We began with the literary survey, which was conducted to find any mention of complications in studies using rabbit hyperbaric oxygen treatment. Then we included observations from two animal experiments done in our department. Our experiments worked with two groups; the first group (n = 24) with 2 hours/day HBOT exposure and the second group (n = 12) with two times 2 hours/day HBOT exposures which provided 384 dives in total. During the experiments, several otoscopy observations were made as well as close video monitoring of the animals.

*Results:* The literature analysis does not provide sufficient direct information on the tolerance of the change in atmospheric pressure or on other complications associated with hyperbaric therapy as such. Our experimental data also did not show pathological changes.

*Conclusion:* Rabbits are probably very tolerant of changing atmospheric pressure. This claim is based on study information and our experiment, but there is still room for a detailed physiological analysis of the problem.

**Key words:** Hyperbaric oxygen. Animal models. Middle ear barotrauma. Compression.

**ABSTRAKT**

*Východiská:* Naše práce informuje o možných komplikacích při hyperbarické kyslíkové terapii (HBOT) u králíků. Hlavním problémem je barotrauma středního ucha, protože jde o nejčastější komplikaci v léčbě lidí. Studie na zvířatech se obvykle zaměřují na požadované cíle, ale málokdy detailně popisují metodologické technické otázky.

*Metody:* Abychom mohli posoudit snášenlivost králíků vůči kompresi, zkombinovali jsme několik nezávislých metod. Začali jsme literárním průzkumem, který byl proveden za účelem nalezení jakékoliv zmínky o komplikacích ve studiích využívajících hyperbarickou kyslíkovou léčbu u králíků. Pak jsme zahrnuli pozorování dvou pokusů na zvířatech, které byly provedeny na našem oddělení. Naše experimenty pracovaly se dvěma skupinami; první skupina (n = 24) s 2 hodinovou expozicí HBOT denně a druhá skupina (n = 12) s dvěma 2 hodinovými expozicemi HBOT za den, které poskytly celkem 384 ponorů. Během pokusů bylo provedeno několik otoskopických pozorování a také podrobné sledování zvířat na videu.

*Výsledky:* Analýza literatury neposkytuje dostatečné přímé informace o toleranci změny atmosférického tlaku ani o jiných komplikacích spojených s hyperbarickou terapií jako takovou. Naše experimentální data také neukázala patologické změny.

*Závěr:* Králíci jsou pravděpodobně velmi tolerantní ke změně atmosférického tlaku. Toto tvrzení je založeno na studijních informacích a našem experimentu, ale stále je zde prostor pro podrobnou fyziologickou analýzu problému.

**Klíčová slova:** Hyperbarický kyslík. Zvířecí modely. Barotrauma středního ucha. Komprese.

## BACKGROUND

The aim of this study was to examine the tolerance to changes in atmospheric pressure during hyperbaric oxygen therapy (HBOT) application in an animal testing. We worked with New Zealand rabbits and found that data on tolerance to pressure changes in animals were very limited or missing. When we started preparing the methodology of our work [1], it was a big problem. Animal studies conducted in a medical context typically do not focus on such details. But we could probably say that if a lot of research was done and there were no references to complications, it probably wouldn't be a very common problem. But if even the veterinary specialist wasn't sure, we decided to take this potential problem into account. To maximize animal safety, we decided before the experiment to conduct literature research specifically on this detail and to take precautions during the experiment itself.

Middle ear barotrauma may seem like an unimportant detail, considering that many studies explore topical topics in order to advance different medical areas. During HBOT, however, middle ear barotrauma is probably the most common complication

– at least in treating people. Up to 17 % of treated patients [2] may suffer from barotrauma. There's no reason to think it could be anything else for a rodent. The wellbeing of experimental animals should be one of the priorities, which is why we questioned the tolerance to pressure changes in rabbits prior to the experiment. The pain caused by eardrum barotrauma can be quite significant – as patients report in their daily routine. After middle ear barotrauma – if left untreated and if HBOT is not interrupted – the situation worsens further in the following days and may lead to complications such as eardrum perforation or *otitis media* [3, 4]. With rabbits undergoing many HBOT sessions every day, this could be a big problem. Even if the risk is the same for any number of subjects, there is probably a greater chance that they will notice problems with repeated daily exposures. We could mention studies with hundreds of hyperbaric exposures without mentioning any complications – 350 [5], or more than 500 [6]. It's not just a question of ethics and pain, but since rabbits are sensitive during experiments, it can affect the experiments by creating an intense stress factor for the animals.

Some studies work in the head area of the rabbit, such as research on irradiated mandibules [7], wounds performed in the ear area [5] and others with surgery in the ENT area [8]. We suggest that there is an even greater risk of middle ear barotrauma in these experiments. Inflammation or change in tissue in the Eustachian tube region leads to increased balancing problems – at least in humans [9]. However, in this case we also did not find the information required.

If we're talking about equalizing middle ear pressure, there's a question of how to even recognize the problem. In animals, we are probably limited to behaviours typical of pain – agitation, rapid movements, noise or aggression [10].

## MATERIALS AND METHODS

Our institute has worked with 36 New Zealand rabbits subjected to pressure changes in total. The primary objective of the research was to observe wound healing at various levels under hyperbaric conditions – gene transcription, histopathology, macroscopy and more. For this purpose, we created a set of four skin wounds on the back of each rabbit. The wounds were 2x2 centimetres long and deep up to the muscle fascia. Further surgical and other details associated with the main research plot are not relevant in this paper as we want to assess the tolerability of pressure changes. The animals were then exposed to hyperbaric treatment. A group of 24 rabbits received one treatment daily for 8 days/sessions and a group of 12 rabbits received two hyperbaric sessions for 8 consecutive days. So we did a total of 384 hyperbaric sessions. During treatment in the chamber, the animals were fully conscious.

### Hyperbaric treatment

We used a modified human hyperbaric chamber (type: ČKD, date of manufacture 1960, factory identification number 5622). Inside the chamber, each rabbit was placed separately in steel, semi-hermetic box with a transparent safety-glass lid. The compartments were designed according to the requirements and recommendations of the American Society for Testing, Materials International and WHA International. Ventilation was ensured with a constant oxygen flow of 4 l per minute. The hyperbaric profile consists of a 15-minute compression at 1.5 ATA with a compression rate of 0.1 ATA/min. There was a 90-minute isobaric session and then a 15-minute decompression.

We watched the rabbits on CCTV, we could see all the treated animals from above in HD resolution. We looked for animal agitation or aggression as a sign of discomfort. The audio signal was also recorded from the chamber.

We planned to do an ongoing tympanoscopy, but found it very complicated because the rabbits did not cooperate. It was impossible without sedatives. The rabbit ear duct is relatively long and requires either specialized devices or a sedated animal. There is also a problem with medical observations – animal pathological anatomy does not have to be directly compared to human.

## RESULTS

We observed no signs of animal discomfort during the experiments. All of the 384 exposures were closed so that no complications were noted. CCTV shows calm animals, some of which lying motionless. The rabbits did not have even examined their compartments and seemed comfortable. After the treatment, we could personally tell that they were calm even as they returned to their cages, perhaps even looking tired. However, this cannot be objectively assessed and is presented only as the opinion of authors (those who have worked with animals). Limited otoscopic observations (see methodology)

did not reveal any suspicion of damage or irritation to the eardrum.

## DISCUSSION

Our experience confirmed the expected results. Rabbit tolerance to equalize middle ear pressure is very high and rabbits are animals commonly used in experiments including hyperbaric exposure. However, there is a degree of uncertainty as the data collected is only indirect. We could probably measure some indicators of stress blood levels, but that would probably be very complicated and also impossible for experiments using hyperbaric exposure only as part of the treatment, not as a goal of research. But we believe the pain caused by the barotrauma would be enough for the rabbit to trigger a defensive reaction and we haven't seen one. As regards the detection of intolerance and discomfort to the individual, the preparation of a sufficient and robust camera system should be encouraged for any animal research.

Extensive literature searches were conducted on the Internet without a finding of intolerance. However, we must stress that this point was not the target of any study. A systematic approach can be complicated, even unfair, because reading a report may not be enough. Addressing "small" complications within the scope of the research itself may be unwanted due to publishing strategies (for example word count).

Routine tympanometry and otoscopy could be considered part of the methodology, but there are also problems. These techniques are standardized for humans and therefore no results can be considered absolutely reliable. Handling the ear canal would also require some sedation of the animal and would likely cause great stress on its own.

Then there is the question of possible steps if we find intolerance in the rabbit. There are not many options. In some trials, tranquilized rabbits [10] are used, but this is more of an exception. HBOT administration is usually repeated and sedation would not be a possible solution. Reducing compression speeds is a potential and very straightforward solution. This can be easy and effective, but there are complications. There may be time constraints or standardization problems. Another option is to "motivate" rabbits to increase the amount of jaw movements. They do it in general, but with some food inside the box, it could be stimulated.

Some studies describe the technical context in a little more detail. The chamber name [6, 12] can be traced to technical parameters. Compression time is also usually indicated. The duration is usually 15 minutes as this corresponds to commonly used human treatment profiles. This may be important, because slower compression may be more bearable if we agree that rabbits do (involuntarily) some of the balancing manoeuvres.

We also found several other technical references – gas concentration and ventilation to space [12] or a rare reference to the design of gas exchange [13], which is also a very useful detail as the ventilation of rabbits is another problem – insufficient quantities can lead to water vapour gathering, which can lead to the problems of observation through transparent walls/windows (if any). Inadequate ventilation would also apparently lead to a reduction in respiratory gas exchange.

A major concern with suspected barotrauma would be the question of continuing hyperbaric therapy. We probably can't interrupt important research on suspicion, but on the other hand, a painful administration would be unethical and could lead to a distortion of the experiment.

## CONCLUSION

Tolerance in rabbits to change middle ear pressure is high and complications are not yet reported. We still believe that a careful evaluation is required in all trials. Detailed descriptions of procedures are recommended, although they may seem plentiful.

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***Adherence to ethical standards***

*The study was approved by the Expert Committee on Experimental Animal Care at the Faculty of Military Health Sciences of the University of Defence. The experiments were carried out in accordance with the guidelines of EU Directive 2010/63/EU on animal experiments and the conditions laid down in the national order on the breeding and use of experimental animals. All workers who have handled animals hold a certificate of professional competence for the design of experiments and experimental experiments under the Animal Welfare Act against Cruelty. The veterinary specialist was part of the research team.*

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