Bratisl Lek Listy 2011; 112 (8)
447 – 452

CLINICAL STUDY

Short term oxidative DNA damage by hyperbaric oxygenation in patients with chronic leg ulcers

Zimanova J[1], Batora I[1], Dusinska M[2,5], Burghardtova K[2], Blazicek P[3], Vojtech I[4], Bizik A[1]

Department of Occupational Medicine and Toxicology, Faculty of Medicine, Comenius University Bratislava, Slovakia. **j.zimzim@gmail.com**

Abstract: *Introduction:* Hyperbaric oxygen therapy (HBO) is successfully used for the treatment of a variety of conditions and diseases. HBO therapy can be valuable for treating selected cases of hypoxic diabetic leg ulcers and chronic venous insufficiency. Exposure to high concentration of oxygen is known to induce damage to cells, possibly due to an increased oxygen radical production. Reactive oxygen species also cause DNA damage.
Method: The Comet assay method has been used to determine DNA damage. Number of DNA strand breaks obtained by the single cell gel electrophoresis in nucleuses of lymphocytes was isolated from venous blood. Nucleuses of lymphocytes were incubated by bacterial repair endonucleases, which detect and remove damaged parts of DNA (SBs, FPG, AlcA, ENDO, and H_2O_2).
Material: 27 patients were investigated in this study; diabetes mellitus was diagnosed in 15 of them, and chronic venous insufficiency in 12. They were exposed in average 27 times in a hyperbaric chamber to a pressure of 2.5-3ATA of 100 % oxygen. Lymphocytes were isolated from venous blood before treatment and at different time after treatment (24 hours, 7 days, 14 days, 6 weeks).
Results and conclusion: Results of DNA damage evaluation at different time periods suggest there are no significant changes if compared to initial DNA damage values. HBO treatment can be used as adjuvant treatment because no significant risk is manifested with this therapy *(Tab. 1, Fig. 7, Ref. 52).* Full Text in free PDF *www.bmj.sk.*
Key words: oxidative DNA damage, hyperbaric oxygenation, chronic leg ulcers.

Healing of lower extremity defects of various origin is based on complex approach and combination of several treatment guidelines resulting from the origin of defect, education, and patient compliance. Standard treatment guidelines often do not lead to the expected effect. Hyperbaric oxygen therapy frequently deployed in last years is one of the methods supporting and accelerating healing effects. Long-term uncertainty and discussions on safety of this treatment lead us to assess its healing effect by monitoring parameters which could better clarify its safety resulting from hyperoxygenation induced at hyperbaric chamber exposition.

Aim of the work was to assess oxidation stress and ROS at short-term damaged DNA influenced by repeated expositions to hyperbaric oxygen therapy in patients with chronic and non-recovering lower extremity defects of various origins.

Chronic ulcers of low extremities have venous, arterial, diabetic, and posttraumatic etiology. The pathophysiologic process of dermal leg defect genesis is a complex process (6, 8).

[1]Department of Occupational Medicine and Toxicology, Faculty of Medicine, Comenius University Bratislava, Slovakia, [2]Slovak Medical University, Bratislava, Slovakia, [3]ALPHA MEDICAL, Bratislava, Slovakia, [4]Department of infection and Geographic Medicine, Faculty of Medicine, Comenius University Bratislava, Slovakia and [5]Norwegian Institute for Air Kjeller Norway

Address for correspodence: J. Zimanova, Dept of Occupational Medicine and Toxicology, Faculty of Medicine, Comenius University, Limbova 1, SK-833 05 Bratislava, Slovakia.

Chronic, not recovering defects and wounds identified as problematic are those not healing, and reacting neither on standard surgical nor other treatment regimens. They are most frequently localized on lower extremities (23, 24, 25). Regardless of the origin their common features are circulation disorders and presence of infections. Clinical surveys and animal experiments proved inhalation of oxygen at high pressure accelerating the recovery of ischemic and infected flaws (7, 8, 44). Hyperbaric oxygen therapy is usually considered when defects and gangrenes do not recover permanent local and global intensive care within weeks and months.

Hyperbaric oxygen therapy (HBO) is a treatment method where patient inhales pure oxygen in pressurized chamber with increased pressure than normal (sea level 1ATA). HBO is not an isolated treatment method, in majority of cases it is a component of complex treatment process (3, 4, 24, 32, 33, 36, 41).

Physiologic principle of the method is derived from the transport role of blood for oxygen, which is being driven to tissues as chemically bound to hemoglobin, or physically dissoluted in plasma. It is known that 1 gram of hemoglobin transfers 1.34 ml of oxygen. The share of physically dissolved oxygen in plasma is very small and at normal circumstances clinically meaningless. It grows to 6.6 volume per cent at pressure of 0.3 MPa. It means that the main importance of HBO is in increasing the physically dissolved oxygen in plasma to such range to totally cover needs for oxygen (3, 6, 36, 41).

Adverse effect of HBO

It is assumed that HBO may invoke a toxic effect by creation of free oxygen radicals. Free radicals are considered risk factors in a number of chronic and degenerative diseases including cancer. Some current publications on a contrary showed that HBO is not connected with an increasing of degradation and of tissues by means of free radicals (26, 27, 49).

Patients and group features

The group was composed of 27 patients with crus defects of various origins, having been repeatedly long-term but unsuccessfully or insufficiently treated by dermatologists, angiologists, or surgeons.

The group of patients with chronic not recovering flaws of lower extremities was further split to 2 subgroups based on the primary diagnosis.

First group consisted was created of patients with Diabetes mellitus type I and II regardless of the compensation of diabetes with existing defect or developed diabetic leg n=15. Patients were sorted by Wagner's classification. They belonged to second to fifth level of damage according to the mentioned classification (7, 8).

Second group consisted of patients with chronic venous insufficiency with crus ulcers n=12. In this case CEAP classification of chronic venous insufficiency was used. Patients in this group belonged to C6 grade of CEAP classification I (7, 8). Venous samples were obtained needed for isolation of peripheral lymphocytes for the purpose of further analysis by means of Comet assay. Lymphocytes were isolated from these samples 24 hours, 7 days, 14 days and 6 weeks after the last exposure in HBO. The umber of hyperbaric exposures was limited by clinical status of the patient and recovery course of the defect. The patients underwent once daily 5 times in a week a serial of hyperbaric exposures (hyperbaric pressure 0.18–0.20 MPa, 72 min netto inhalation time with 100 % oxygen). The duration of the whole exposure was in total 120 min. Treatment effect was assessed at the end of week 6 by percentage expression of dermal defect change of the area.

Methods and materials

Comet assay (simple cellular alkali gel electrophoresis) is a sensitive method to detect fragments of DNA, oxidized purines and oxidized pyrimidine in individual cells (5, 6, 7, 8, 22).

This method is based on the ability of single chain DNA to liberate from superhelix. Enzyme formamidepyrimidine DNA glycosylase (FPG) is used for detecting oxidized purines, and for detection of oxidized pyrimidins endonuclease III (ENDO) is used in Comet assay. Alkylating damage of DNA is detected via 3-metyladenin DNA glycosylase II (AlcA) (14, 15, 16, 20). Sensitivity to H_2O_2 is observed. Oxidative impact of H_20_2 induces changes which determine to what extend is the cell able to repair oxidative damage of DNA. It is assumed that the more the DNA is damaged, the less is the repairing ability of the cell against oxidative damage.

Tab. 1. The statistical difference equal in comparison with the subgroup of diabetics.

Parameter	N	Mean area [cm^2] before HBO	Mean area [cm^2] after	Sign
total	23	2140 [0,5–180,25]	13,82 [0–154,75]	0,014
DM	13	12,58±10,67	2,87±2,90	0,003
CVI	10	32,88±57,21	28,05±49,69	NS

Legend: DM – diabetes mellitus, CVI – chronic venous insufficience

Results

Average number of exposures in hyperbaric chamber was 27, ranging from 14 to 32. ubgroup of diabetics involved 15 patients, subgroup with CVI 12 patients.

The set of patients was represented with nonsmoking males 92 %, only minimum 8 % [n=2] were smokers. Almost half of the patients 42 % [n=11] suffered from obesity, 31 % [n=9] were overweight, 27 % [n=7] had normal weight. Out of the male patients 69 % [n=11] had DM and 31 % [n=4] had CVI. 64 % of females [n=7] had CVI and 36 % [n=4] had DM.

The defect area before the onset of HBO was 21.4 cm^2 in average. The largest defect reaching 180 cm^2 was observed in a female patient from the subgroup with CVI. After finishing HBO the defect area was reduced in average by 8.0±10.1 cm^2, whereas in 4 patients total healing was achieved. After finishing HBO and evaluation of the defect areas in all patients the area reduction was 62.2±36.1% in average. The subgroup with DM reduced the defect area in average by 70.3±27.3% i.e. by 9.7±9.8cm^2. The subgroup with CVI reduced the defect area only by 51.8±44.5% i.e. 5.8±10.7cm^2. This difference was statistically equal in comparison with the subgroup of diabetics (Tab. 1). The defect size in group with DM was already before deployment of HBO smaller than in the group with CVI patients. There were remarkable differences in size of the defects and the set showed non-Gaussian distribution of the defect size.

Parameters of oxidative damage of DNA

Average values of oxidative damage SBS – basic DNA strand breaks were measured in arbitrary units. Average value of input measurement before HBO was 209.1±90.6 arbitrary units. Measurements in particular time slots showed only insignificant oscillations of mild increase of DNA strand breaks in period from first 24 hours till day 14. These values dropped after 6 weeks since last HBO exposure. Similar course was observed at both groups with DM and CVI. These differences were not significant (Fig. 1).

Average values of further DNA damage, in this case on purine bases detected by FPG enzyme, were 263.7±101.5 arbitrary units before onset of HBO. Similarly as at SBS we recorded

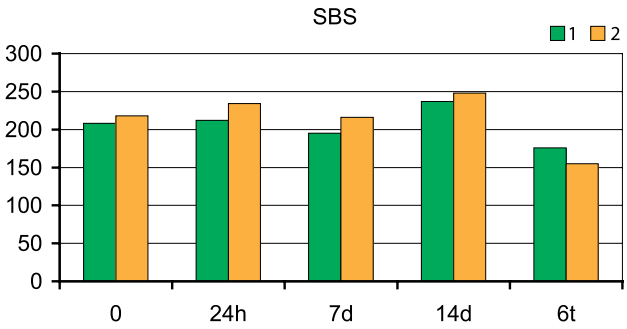

Fig. 1. The course and changes in SBS in time and both patient groups. Legend: SBS-0 – damage in time 0 = 0 hours, 24h = 24 hours, 7d = 7 days, 14d =14 days, 6t = 6 week after last exposure, 1 = Diabetes mellitus, 2 = Chronic venous insufficiency.

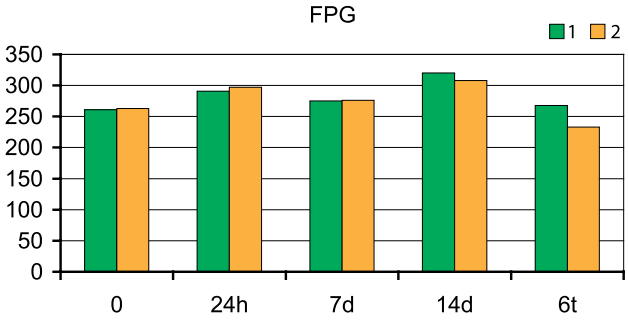

Fig. 2. The course and changes in SBS-FPG time both patient groups. Legend: S-FPG-0 – damage on purine bases in time 0 = 0 hours, 24h = 24 hours, 7d = 7 days, 14d = 14 days, 6t = 6 week after last exposure, 1 = Diabetes mellitus, 2 = Chronic venous insufficiency.

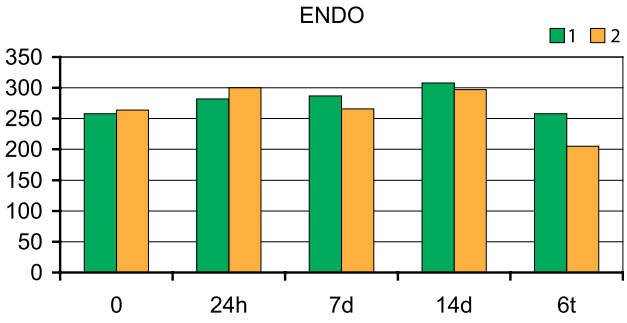

Fig. 3. The course and changes in SBS-ENDO time both patient groups. Legend: S-ENDO-0h – damage on purine bases in time 0 = 0 hours , 24h = 24 hours, 7d = 7 days, 14d = 14 days, 6t = 6 week after last exposure, 1 = Diabetes mellitus, 2= Chronic venous insufficiency.

growth dynamics in the time of sample uptake after 24 hours, 7 and 14 days after finishing HBO. This effect was no more observed at week 6, paradoxically it moved to the initial value. Differences were not statistically significant (Fig. 2).

A similar trend and dynamics were observed when determining oxidative damage values of oxidized pyrimidine detected by endonucleasis III – ENDO (257.1±89.6 arbitrary units), same as alkylating DNA damage detected by glycosylase II where average values (AlcA) were 269.5±87.2 arbitrary units (Figs 3, 4).

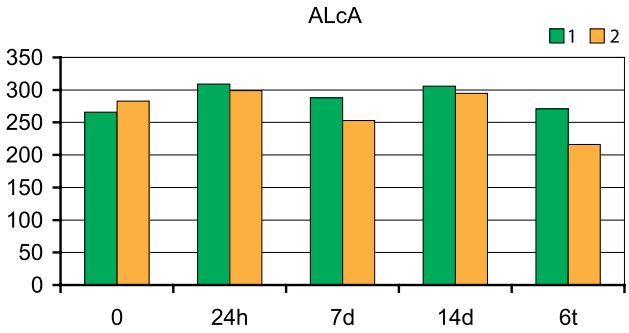

Fig. 4. The course and changes in SBS-ALcA time both patient groups. Legend: NET-ALCA-0h – damage on purine bases in time 0 = 0 hours, 24h = 24 hours, 7d = 7 days, 14d = 14 days, 6t = 6 week after last exposure, 1 = Diabetes mellitus, 2 = Chronic venous insufficiency.

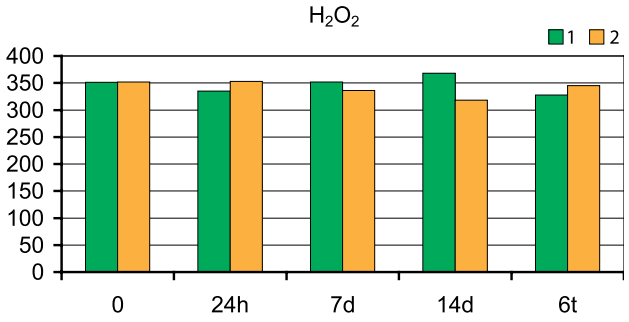

Fig. 5. The course and changes in SBS-H_2O_2 time both patient groups. Legend: S- H_2O_2 -0h – damage on purine bases in time 0 = 0 hours, 24h = 24 hours, 7d = 7 days, 14d = 14 days, 6t = 6 week after last exposure, 1 = Diabetes mellitus, 2 = Chronic venous insufficiency.

Average values of oxidative damage of DNA by hydrogen peroxide (further H_20_2) were 350.6±39.9 arbitrary units. Measurements in particular time slots showed only insignificant oscillations of mild increase to day 14, with recorded drop at week 6. The course in both groups was similar. Oxidative increase in the patient group with DM was higher, but statistically not significant (Fig. 5).

Due to a higher number of followed parameters in our survey we performed a correlation analysis to determine how certain factors associate with changes in the size of the defect.

Correlation of anthropometric data such as weight, height, BMI, waist circumference, then age of the patient with changes in defect size, with changes and course of oxidative damage in certain monitored parameters and time spans, statistical contexts were not confirmed.

A strong correlation was confirmed between values of SBS ENDO damage and FPG damage of DNA, in reciprocal proportion with the defect size, which might lead to assumption that the higher are the damages, the worse is the healing of the defect.

Discussion

Hyperbaric oxygen therapy is broadly deployed mainly in the last 30 years. In the treatment of hypoxia and ischemia in defects of lower extremities by means of HBO its important

effects such as stimulation of fibroblastic proliferation and increased collagen formation, extension of new vascularization, and stimulation of bactericidal effect of lymphocytes are used (37, 45). Group of authors confirmed a positive effect in healing of the defects and salvage of the limbs. They described a reduction of amputations, better recovering after amputation with the use of HBO as one of the adjuvant treatment methods (7, 8, 11, 12, 29, 34, 52). We have less information about HBO effect on healing defects of non-diabetic origin caused mainly by CVI, but the positive effect is doubtless (30, 51). Roeckl-Wiedman et al (40) described a significant reduction of defect size. Bongiovanni et al (9) added significant shortage of ulcus healing period. Performing further surveys is necessary for explicit confirmation of HBO usefulness in the treatment of crus defects having venous etiology (40).

On the other hand there are studies assigning treatment with hyperbaric oxygen not such unequivocal effect (13, 50). They notify on unsatisfactory and unsoundly determined sorting criteria, various defect origins, unsatisfactory control groups, and insufficient assessment of accompanying diseases, next supportive treatment, or other factors which could positively or negatively influence the final effect of healing defects (50). As described by Doležal et al, aside the medical aspects the pharmacoeconomic effect and economic benefit of this therapy is also important (23, 25).

Bátora et al tried to present a good effect of HBO in treatment of defects caused by DM-DN and CVI in a limited group comparable with other surveys. A significant defect area was achieved in both groups. Success in group of diabetics was more remarkable (7, 8). Moreover a significantly faster healing was confirmed with diabetics compared to standard treatment methods. The effect was statistically significant, however not so visible in group with CVI defects (8). The mentioned surveys followed the effect of HBO on defect recovery.

Because of known effects of hyperbaric oxygen therapy a set of surveys was created to deal with monitoring of side effects as creation of ROS, and related oxidative DNA damage and genotoxicity (1, 28, 35).

In the course of last years a context of oxidative stress with DNA damage was proven, with a possible molecule transcription followed by occurrence of degenerative diseases such as atherosclerosis, cancer, neurodegenerative diseases, Alzheimer disease, inflammatory joint diseases, diabetes mellitus, and others (1, 28, 35). Oxidative stress as a factor of several diseases is a cause as well as an effect (28).

Fig. 6. HBO chamber for 6 persons in Dept. of Occupational Medecine and Toxicology Bratislava 2004.

Comet assay is a method of investigation and measuring damaged parts of DNA, and its repair, or monitoring mutagenic environment effects in population (18, 19, 31, 39, 47). This method is broadly deployed in genotoxic tests, which include enzyme peculiarity for various lesions, such as oxidized pyrimidines and purines in DNA molecules. They represent sites of disconnection of damaged DNA, which are recognizable with reparative endonucleases (17, 18, 19). Thus it is possible to unhide significant correlations between oxidized DNA bases associated with oxidative stress (17, 18).

All results from mentioned experiments and surveys were gained and processed in normobaric conditions. Hence the next set of experiments focused on further potential sources of oxidative damage. It was proved that using conditions of HBO as one of the free oxygen radical sources (e.g. exposure to 100 % oxygen and pressure of 2,5 ATA) lead to a clear multiplication of DNA strand breaks detectable by Comet assay in leukocytes of human subjects (21, 42, 43, 48, 49). Finally a concluding assumption of the performed surveys was that the oxidative stress after HBO causes oxidative DNA damage which lasts just a certain time period (21, 23, 42, 49).

Common denominator of those surveys was research performed with healthy volunteers.

Our survey was focused on both lines observed in currently performed monitoring. Successful healing effect of HBO on defects of mainly diabetic, ischemic, and CVI etiologies, thus in populations with already developed chronic disease being itself

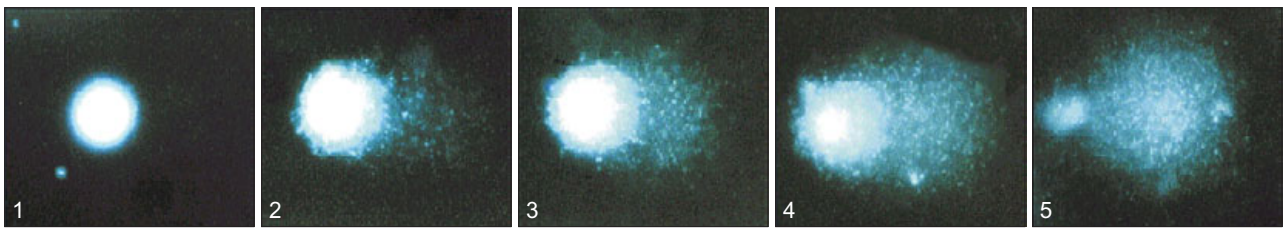

Fig. 7. Comet Assay – DNA damage grades: 1. healthy nucleus; 2–4. extending damage, 5. fully damaged nucleus.

a source of oxidative damage products (34) on the one hand, bearing in mind side effects of HBO, where dominant effect is that oxidative damage, with the aim to identify eventual relationship that could influence both monitored aspects.

The group of our patients with diagnoses of DM or CVI by defect etiology was given hyperbaric oxygen therapy, and measurement of oxidative DNA damage was performed 24 hours after last exposition in HBO, then after 7 days, 14 days, and in some patients also in week 6 after HBO. We recorded similarly to other surveys in given time periods (21, 22, 42, 48) only mild insignificant oxidative damage increase which in all examined parameters lasted up to 14 days after HBO. Levels of oxidative damage after 6 weeks dropped almost to the level of baseline values observed in patients before start of HBO.

Statistically significant negative correlation was confirmed for SBS ENDO damage of DNA and FPG damage itself with difference in defect size which results in assumption that the larger is the damage the worse is the healing. The healing effect of HBO was unsatisfactory in some patients, whereas increased number of breaks with damage on pyrimidine and purine bases was observed even before HBO. In course of HBO, and in given time periods after HBO nothing changed statistically significantly. This might have been a string in patient selection for HBO therapy, eventually their better split to groups with a good feedback to HBO vs. group with insufficient reaction and need for a longer HBO deployment, which opens an issue of higher risk of DNA oxidative damage. It is to consider if deployment of this form of treatment in this patient group has its justification, and at the same time raises the question of collateral intensive antioxidant support during HBO therapy with prolonged exposure (2, 37, 46, 47, 48).

mall number of patients was due to closure ofn the HBO unit in Bratislava, Clinic for Occupational Diseases and Toxicology, and disabled extension of patient group (Figs 6, 7).

Conclusion

1) Detection of DNA oxidative damage level proved only insignificant increase of oxidative DNA damage in separated parameters with a trend to drop in week 6 after last hyperbaric exposure.

2) Statistically significant differences in oxidative damage were not observed in patients split in groups by diagnoses of DM and CVI.

3) Because the increased DNA oxidative damage is statistically insignificant after repeated HBO deployment also in of chronic inflammatory diseases, this method of treating unrecoverable defects of extremities is a suitable supplementary and additional method to manage those situations, and should be part of the treatment.

4) HBO can remarkably contribute to shortening the length of hospital stay, reduce morbidity and mortality of patients with crus defects of various origin

5) Our work although thin in range without prejudice confirmed a good effect of HBO for treatment of defects in patients with DM and CVI with substantial reduction effect of the defect area, acceptable for the reviewers of the method. As a part of a complex therapy in position of effective additonal treatment has a sustained place as a safe treatment method (7, 8, 10, 23, 24, 25).

References

1. Anderson D, Yu TW, Wright J, Ioannides C. An examination of DNA strand breakage in the comet assay and antioxidant capacity in diabetic patients. Mutat Res 1998; 398 (1–2): 151–161.

2. Bader N, Bosy-Westphal A, Koch A, Mueller MJ. Influence of vitamin C and E supplementation on oxidative stress induced by hyperbaric oxygen in healthy men. Ann Nutr Metab 2006; 50 (3): 173–176.

3. Barcal R, Emmerová M, Hadravský M. Hyperbarie a hyperbarická oxygenoterapie. Plzeň: Vydavatelství J. Zuna, 2000. 122 s.

4. Barcal R, Emmerová M, Hadravský M. Základy hyperbarickej medicíny. Praha: Univerzita Karlova, 1998, 203 s.

5. Barnett YA, King CM. An investigation of antioxidant status, DNA repair capacity and mutation as a function of age in humans. Mutat Res 1995; 338: 115–128.

6. Bátora I, Bátorová A, Bízik A, Varga A. Chronic venous ulcer in patients with inhereted APC resistance – successful treatment with hyperbaric oxygen. Haemostasis 2000; 30 (Suppl 1): 113.

7. Bátora I, Bízik A, Bátorová A. Hyperbarický kyslík v liečbe vredov predkolenia (1. kongres nemocí z povolání s medzinárodní účasti. Luhačovice, 15.–16.11.2002). Prac Lék 2002; 54 (4): 165–166.

8. Bátora I, Zimanová J, Uličná O, Vojtech I et al. Efekt hyperbarickej oxygenoterapie v liečbe chronických nehojacich sa vredov dolných končatín. Prac Lék 2005; 57 (2): 66–72.

9. Bongiovanni CM, Hughes MD, Bomengen RW. Accelerated wound healing multidisciplinary advances in the care of venous leg ulcers. Angiology 2006; 57 (2): 139–144.

10. Buchancová J, Klimentová G, Gáliková E. Novinky v pracovnom lekárstve a toxikológii. Martin: Jesseniova lekárska fakulta univerzity Komenského, 1998, 102 s.

11. Cianci P. Advances in the treatment of the diabetic foot: Is there a role for adjunctive hyperbaric oxygen therapy: Wound repair and regeneration 2000; 12 (1): 2–10.

12. Cianci P, Petrone G, Drager S, Lueders H, Lee H, Shapiro R. Slavage of the problem wound and potential amputation with wound care and adjunctive hyperbarix oxygen therapy: an economic analysis. In: Cianci P. Advances in the treatment of the diabetic foot: Is there a role for adjunctive hyperbaric oxygen therapy: Wound repair and regeneration 2000; 12 (1): 2–10.

13. Ciaravino ME, Friedell ML, Kammerlocher TC. Is hyperbaric oxygen a useful adjunct in the management of problem lower extremity wounds? Ann Vasc Surg 1996; 10 (6): 558–562.

14. Collins AR. Oxidative DNA damage, antioxidants and cancer. BioEssays 1999; 21: 238–246.

15. Collins AR, Cadet J, Epe B, Gedik C. Problems in the measurement of 8-oxoguanine in human DNA. Report of workshop, DNA Oxidation, held in Aberdeen, UK. Carcinogenesis 1997; 18: 1833–1836.

16. Collins AR, Dobson VL, Dušinská M, Kennedy G, Štetina R. The commet assay:what can int really tell us? Mutat Res 1997; 375: 183–193.

17. Collins AR, Dušinská M, Franklin M, Somorovská M, Petrovská H, Duthie S, Fillion L, Panayiotidis M, Rašlová K, Vaughan N. Comet assay in human biomonitoring studies reliablilty, validation and applications. Envirom Mol Mutagen 1997; 30 (2): 139–146.

18. Collins AR, Dušinská M, Gedík CM, Štetina R. Oxidative damage to DNA: Do we have a reliable biomarker? Enviromental Health Perspectives 1996; 104 (Suppl 3): 465–469.

19. Collins AR, Duthie SJ, Dobson VL. Direct enzymic detection of endogenous opxidative base damage in human lymphocyte DNA. Carcinogenesis 1993; 14 (9): 1733–1735.

20. Collins AR, Ma A-g, Duthie SJ. The kinetics of repair of oxidative DNA damage (strand breaks and oxidized pyrimidines) in human cells. Mutat Res 1995; 336: 69–77.

21. Dennong C, Hartmann A, Frey G, Speit G. Detection of DNA damage after HBO therapy. Mutagenesis 1996; 11: 605–609.

22. Dennong C, Rademacher P, Barnett ZA, Speit G. Antioxidant status in humans after exposure to hyperbaric oxygen. Mutat Res 1999; 16 (1–2): 83–89.

23. Doležal V. Hyperbarická oxygenoterapie nehojících se ran a defektů. Čas Lék čes 2001; 140 (4): 104–107.

24. Doležal V. Hyperbarická oxygenoterapie. Praha: Academia, 1995, 420.

25. Doležal V. Možnosti léčby diabetické nohy hyperbarickým kylsíkem. Prakt Lék 2001; 81 (3): 129–130.

26. Ďuračková Z. Voľné radikály a antioxidanty v medicíne I. Bratislava: Slovak Academic Press, 1998, 285.

27. Ďuračková Z, Bergendi Ľ et al. Voľné radikály a antioxidanty v medicíne II. Bratislava: Slovak Academic Press, 1998, 306.

28. Dušinská M, Rašlová K, Lietava J, Somorovská M, Petrovská H, Dobríková P, Collins A. Elevated DNA damage in lymfocytes from ankylosing spondylitis patients. Natural antioxidant and anticarcinogens in Nutrition, Health and Diseases, 1999: 433–436.

29. Duzgun AP, Satir HZ, Ozozan O, Saylam B, Kulah B, Coskun F. Effect of hyperbaric oxygen therapy on healing of diabetic foot ulcers.: J. Foot Ankle Surg 2008 Nov–Dec.: 47 (6): 515–519.

30. Hammarlund Ch, Sundberg T. Hyperbaric oxygen reduced size of chronic leg ulcers: A randomiyed double-blind study. Plast Reconstr Surg 1994; 4: 829–833.

31. Horváthová E, Slameňová D, Hlinčiková L, Manda KT, Gábelová A, Collin AR. The nature and origin of DNA single-strand breaks determined with the comet assay. Mutat Res 1998; 409: 163–171.

32. Jain Kewal K. Textbook of hyperbaric medicine. Seattle: Hogrefe and Huber, 1999, 256.

33. Krutý F. Využitie hyperbarickej oxygenoterapie. Zdrav Noviny 1997; 46 (16): 8.

34. Lee SS, Chen CY, Chan YS, Chao EK, Ueng SW. Hyúperbaric oxygen in the treatment of diabetic foot infection. Changgeng Yi Xue Za Zhi 1997; 20 (1): 17–22.

35. Mendoza-Nunez VM, Sanchez-Rodriguez MA, Retana-UgaldeR, Vargas-Guadarrama LYA, Altamirano L. Total antioxidantlevels, gender and age as risk factors for damage in lymphocytes of the elderly. Mech Ageing Dev 2001; 122 (8): 835–847.

36. Moricová Š, Krutý F. Využitie hyperbarickej oxygenoterapie v medicínskej praxi. Recipe 1997; 4 (4): 114.

37. Nagyová A, Krajčovičová-Kudláčková M, Horská A, Smolková B, Blažíček P, Rašlová K, Collins A, Dušinská M. Lipid peroxidation in men after dietary supplementation with a mixture of antioxidant nutrients. Bratisl Lek Listy 2004; 105 (7–8): 277–280.

38. Niinikoski JH. Clinical hyperbaric oxygen therapy, wound perfusion and transcutaneous oxymetry. World J Surg 28 (3): 307–311.

39. Plafki C, Peters P, Almeling M, Welslau W, Busch R. Complication and side efects of hyperbaric oxygen therapy. Aviation Space Environ Med 2000; 71 (2): 119–124.

40. Roeckl-Wiedmann I, Bennett M, Kranke P. Systematic review of hyperbaric oxygen in the management of chronic wounds. Br J Surg 2005; 92 (1): 24–32.

41. Roth RN, Weiss LD. Hyperbaric Oxygen and wound healing. Clin Dermatol 1994; 12: 141–156.

42. Rothfuss A, Dennong C, Speit G. Adaptive protection against the induction of oxidative DNA damage after HBO. Carcinogenesis 1998; 19 (11): 1913–1917.

43. Rothfuss A, Merk O, Radermacher P, Speit G. Evaluation of mutagenic effects of hyperbaric oxygen (HBO) in vitro, II. Induction of oxidative DNA damage and mutations in the mouse lymphoma assay. Mutat Res 2000; 471: 87–94.

44. Rušavý Z, Lacigová S, Čechurová D, Jankovec Z, Těšínský P, Emmerová M, Třeška V. Komplexní léčba syndromu diabetické nohy. Prakt Lék 2002; 82 (2): 106–108.

45. Senior C. Treatment of diabetic foot ulcers with hyperbaric oxygen. J Wound Care 2000; 9 (4): 193–197.

46. Speit G, Bonzheim I. Genotoxic and protective effects of hyperbaric oxygen in A549 lung cells. Mutagenesis 2003; 18 (6): 545–548.

47. Speit G, Dennong C, Eichhorn U, Rothfuss A, Kaina B. Induction of heme oxygenese-1 and adaptive protection against the induction of DNA damage after hyperbaric oxygen treatment. Carcinogenesis 2000; 21 (10): 1795–1798.

48. Speit G, Dennog C, Lampl L. Biological significance of DNA damage induced by hyperbaric oxygen. Mutagenesis 1998; 13 (1): 85–87.

49. Speit G, Dennong C, Radermacher P, Rothfuss A. Genotoxicity of hyperbaric oxygen. Mutat Res 2002; 512 (2–3): 111–119.

50. Wang C, Schwaitzberg S, Berliner E, Zarin DA, Lau J. Hyperbaric oxygen for treating wounds: a systemic review of the literature. Arch Surg 2003; 138 (3): 272–279.

51. Wattel F, Mathieu D, Coget JM, Bilard V. Hyperbaric oxygen therapy in chronic vascular wound management. Angiology J Vasc Dis 1990; 1: 59–65.

52. Zamboni WA, Wong HP, Stephenson LL, Pfeifer MA. Evaluation of hyperbaric oxygen for diabetic wounds: A prospective study. Undersea Hyperbaric Med 1997; 24 (3): 175–179.

Received March 15, 2010.
Accepted April 4, 2011.