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# Hyperbaric oxygen therapy and piracetam decrease the early extension of deep partial-thickness burns

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*During the first 24 h, a progression of the burn wound in histological depth or extension is often noted. This can only partially be prevented by the routinely used protocols of fluid resuscitation and burn wound dressing. In a rat model of 5% TBSA burn, hyperbaric oxygen therapy (HBOT) and piracetam were evaluated for their ability to further prevent this early deepening of the burn wound. After infliction of the burn wound, the animals were treated with an accepted basic burn wound treatment consisting of mafenide 10% solution humid dressings. They were then randomized into three groups: a control group (n = 10), receiving no other treatment, a HBOT group (n = 17), receiving 60 min of HBOT (203 kPa) twice daily, and a piracetam group (n = 19), receiving piracetam (200 mg/kg IM) twice daily. On the third day of treatment, the entire burn wound was exised and examined histologically. It was found that both HBOT and piracetam had statistically significant effects on the preservation of epidermal basal membrane ($P < 0.001$ and $P < 0.01$, respectively). HBOT, but not piracetam, further had significant effects on the destruction of skin appendages ($P \leq 0.05$ and $P > 0.05$, respectively) and on the degree of subepidermal inflammation, as measured by leucocyte infiltration ($P < 0.001$ and $P > 0.05$, respectively). Furthermore, the HBOT group showed significantly less leucocyte infiltration than the piracetam group ($P < 0.01$). It was concluded that, although the clinical importance of the small effects on skin appendage and basal membrane preservation may be questionable, the effect on subepidermal leucocyte infiltration is striking and warrants further investigation of the anti-inflammatory effects of HBOT and possibly piracetam. Copyright © 1996 Elsevier Science Ltd for ISBI.*

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## Introduction

Since the original description by Jackson in 1953[1], it is generally accepted that a cutaneous burn wound consists of three distinct zones, each with its own histophysiological characteristics: a central zone of coagulation necrosis, surrounded by a zone of established oedema and capillary stasis, which is in turn surrounded by a zone of active oedema formation. Another related concept, that of a progressive capillary stasis, reversible if dehydration of the burn wound is prevented, was described by Zawacki in 1974[2].

It is, however, a very common observation in serious burn patients that areas that seemed to be 'partial-thickness' burns have to be regraded the next day as 'full-thickness', despite optimal fluid resuscitation and burn wound coverage[3]. This progressive necrosis of tissue cells is closely linked to the degree of oedema formation. Oedema precedes capillary stasis, which in turn provokes capillary sludge by 'rouleaux' formation of the red blood cells and finally capillary thrombosis. The end-point is tissue hypoxia, ischaemia and cell death. Several therapeutic measures have been proposed to prevent this cascade. Some are widely accepted, such as early wound coverage and optimal osmotic vascular filling. Other drugs and physical measures have not gained wide acceptance.

The purpose of this study was to investigate whether hyperbaric oxygen therapy (HBOT) or piracetam, associated with a classically accepted burn wound treatment regime, are able to limit the extent of this ongoing tissue damage during the early phase of burn injury.

HBOT is an effective means of augmenting the oxygen content of arterial blood. During HBOT administration at 203 kPa, $Pa_{O_2}$ values as high as 189 kPa can be obtained, resulting in the physical dissolution of considerable quantities of oxygen in the plasma (Henry's Law). This hyperoxia induces a generalized arteriolar vasoconstriction, without impairing the oxygen delivery to the tissues. Transcutaneous and intratissue measurements of $P_{O_2}$ in the limbs of patients undergoing HBOT, reveals that the peripheral oxygen delivery can be more than ten-fold higher than in normobaric 100% oxygen breathing[4].

Pirecetam is a pharmacological substance, widely used in the treatment of cerebral vascular insufficiency, but also, because of its rheological properties, in the early phase after free tissue grafts with vascular microanastomosis, and in the treatment of frostbite.

## Materials and methods

The experimental model developed by Kaufman et al.[5] has been used. It has been shown to reproducibly create a partial-thickness burn wound of

approx. 5 per cent TBSA, progressing to a full-thickness wound if desiccation of the wound is not prevented. It also has a very low intrinsic mortality rate. A few adaptations have been made, resulting in the following experimental protocol described below.

**Experimental animal**
Female Wistar rats of body weight 200 g (±20 g) are used. The animals are housed in standard cages, submitted to a 12 h light/dark cycle and fed with standard rat chow and water ad libitum. Twenty-four hours before the test, the animals are shaved circumferentially on the abdomen and back, and carefully depilated with anti-allergic thioglycolate cream.

**Burn wound**
Three aluminium cylinders, of exact dimensions (diameter 3.76 cm, height 3.78 cm; total weight 500 g) are placed in a hot water-bath (75°C) for at least 1 h before the beginning of the tests. The animal is anaesthetized with Hypnorm (fentanyl citrate 0.315 mg/ml + fluanisole 10 mg/ml) 0.15 ml/100 mg body weight IM. This dosage provides good anaesthesia for approx. 3.5 h.

The rat is then taken in the left hand, with the right flank exposed. One of the cylinders is taken out of the water-bath, and is placed immediately on the exposed skin for 10 s. No supplemental pressure is applied. The application time is measured with a chronometer. However, neither extra pressure nor a prolongation of the application time would have had a significant influence on the thermal energy transfer to the skin[5]. Surface temperature measurements are performed at the surface of the cylinder (Therm 2285-2, Enginel, Brussels), before and after each application, and show temperatures of 74±1°C before, and 69±2°C after the application. The three cylinders are used alternatively, so that each cylinder is allowed a rewarming time of about 10 min.

In a series of preliminary experiments, biopsies have shown that this method results in a uniform deep partial-thickness burn wound, progressing, over a 24–36 h period, to full thickness even when moist dressings are applied.

**Burn wound care and resuscitation**
Immediately after burn injury, the wound is covered with a dry sterile gauze and the animal is left for 4 h without any treatment.

Then, two punch biopsies (2 $mm^2$) are taken from standardized parts of the burn wound, and a wound dressing is applied, consisting of a sterile gauze, impregnated with 10 per cent mafenide solution. The dressing is covered with an impermeable membrane (Opsite), and with two layers of adhesive elastic circular bandage (Tensoplast). No fluid resuscitation is given.

**Ancillary treatment**
The burn wound dressing is changed daily, under light anaesthesia (Hypnorm 0.05 ml/100 g IM).

The animals are randomized into three groups, by an independent person, not present at the time of burn wound infliction. The control group receives no ancillary treatment. The 'piracetam group' is given 200 mg/kg piracetam IM (Nootropil, UCB Pharma, Belgium), twice daily (12 h interval), the first injection being given 4 h after the burn.

The 'HBOT' group is submitted to hyperbaric oxygen for 60 min, every 8 h the first day, then every 12 h the following 2 days. The first treatment is given 4 h after the burn.

**Hyperbaric oxygen therapy**
HBOT is performed in a small experimental hyperbaric chamber of 60 litres. The animals are placed with their cage inside the chamber, which is, after hermetic closure of the door, flushed with 100 per cent oxygen for 5 min.

Then, over a 5-min period, the chamber is pressurized with 100 per cent oxygen to 203 kPa (absolute pressure). After a plateau phase of 1 h at this pressure, ensuring a constant ventilation with 100 per cent oxygen (to prevent $CO_2$ build-up), the chamber is depressurized over a 5-min period.

This HBOT protocol is similar to the generally accepted protocols in the treatment of burns in the human patient.

**Morphological assessment**
Each animal is weighed daily, before treatment.

The punch biopsies, taken on day 1, are immediately fixed in Bouin's solution for 1 h, then in a 15 per cent formaldehyde solution.

At the end of the study period, the animals are killed by ether inhalation and the burn wound is excised entirely, to the fascia. A small (1 $cm^2$) flap of unburned skin is included in the excision at the proximal end of the wound, to serve as an intra-individual control. The excised wound is fixed in Bouin's solution for 1 h, then in a 15 per cent formaldehyde solution.

After fixation, three histological preparations are made. The first one is a horizontal cut through the middle of the entire specimen and the flap of unburned skin. The other two are horizontal cuts through each of the remaining parts.

Standard haematoxylin-eosin staining is performed, and the specimens are assessed by an independent pathologist, experienced in the examination of burn wound specimens, in the following way:

1. Punch biopsies: a global histological appreciation is made, classifying the specimens as first, second or third degree burn, according to the standard histological criteria of epidermal necrosis, subepidermal dehiscence and skin appendage necrosis.
2. Excised burn wound:
   — *Reference skin flap*: the number of skin appendages per microscopic field (×100) is counted and is considered an indicator of the general type of skin.
   — *Burn wound*: four microscopic fields (×100) are analysed in the central horizontal preparation, and two more in the upper and lower part of the burn wound (six fields in total). In each microscopic field, the following parameters are evaluated:
      a. Degree of destroyed skin appendages, criteria for destruction being: presence of

round or pyknotic cells, disappearance of hair follicle roots, abnormal coloration of the cytoplasm.

b. Degree of destruction of the epidermal cover, with estimation (in $n/4$) of the integrity of the basal epidermal cell layer.

c. Degree of inflammation: per microscopic field, assignment of a score: 0 (no dermal nor hypodermal inflammation), 1 (moderate inflammation, leucocyte presence concentrating around hair follicles) or 2 (severe inflammation, abundant leucocytes present in all skin layers).

**Statistical analysis**

For each animal, a global score for all six microscopic fields is calculated for each of the three histological parameters, by adding the scores for each microscopic field. After dividing by 6, a 'mean' score is obtained for each animal. The number of destroyed skin appendages is further converted to a 'percentage of destruction', by dividing this score by the number of appendages in normal adjacent skin (the 'reference skin flap'), and multiplying by 100.

The means of per group are then calculated, and the groups are compared using a one-way analysis of variance (ANOVA) with Bonferroni correction for the presence of three groups; the null hypothesis being the similarity of all groups.

## Results

Fifty rats have entered the study. Early mortality was 4/50 (8 per cent). No exact cause of the deaths could be given; however, anaphylaxis due to the Hypnorm injection could not be excluded, since one of the animals died even before the depilation could be performed. The 46 remaining animals were randomized, after the burn wound, into three groups: control group ($n=10$), piracetam group ($n=19$) and HBOT group ($n=17$).

The evolution of body weight is given in *Table I*. No statistical differences are observed between the three groups.

*Analysis of the excised normal skin flaps* does not show any significant difference between the three groups (*Table II*).

All *punch biopsies on day 1* have been classified as 'superficial partial thickness wound', with oedema formation, subepidermal dehiscence, and preservation of appendages to the superficial third of the dermis.

*Percentage of destroyed skin appendages on day 3*: in both experimental treatment groups, markedly less skin appendages are destroyed (*Table III*).

**Table I.** Weight loss

| | *Day 1 (g)* | *Day 3 (g)* | *Difference (%)* | *s.d. (%)* | P |
|---|---|---|---|---|---|
| Control | 193.8 | 200.3 | +3.35 | 5.443 | |
| Piracetam | 201.2 | 204.0 | +1.42 | 3.681 | >0.05 |
| HBOT | 198.0 | 201.8 | +1.92 | 4.198 | >0.05 |

Piracetam vs. HBOT: $P>0.05$.

**Table II.** Normal skin analysis

| | *n=5* | *n=6* | *n=7* | *Total* | *Mean* | *s.d.* | P |
|---|---|---|---|---|---|---|---|
| Control | 1 | 6 | 3 | 10 | 6.20 | 0.632 | |
| Piracetam | 4 | 7 | 8 | 19 | 6.21 | 0.787 | >0.05 |
| HBOT | 6 | 5 | 6 | 17 | 6.00 | 0.866 | >0.05 |

Piracetam vs. HBOT: $P>0.05$.

For the HBOT group, the difference is statistically significant (0.61 vs. 0.73, $P<0.05$), but not for the piracetam group (0.64 vs. 0.73, $P>0.05$). The difference between the piracetam group and the HBOT group does not attain statistical significance ($P>0.05$).

*Destruction of the epidermal basal layer (Table IV)*: in both treatment groups significantly less destruction has taken place. The HBOT group yields the highest level of significance (control 0.87; HBOT group 0.73, $P<0.001$; piracetam group 0.78, $P<0.01$). The difference between the piracetam and HBOT groups is not significant ($P>0.05$).

*Degree of dermal and subepidermal leucocyte infiltration (Table V)*: the difference in leucocyte infiltration is not significant for the piracetam group (0.60 vs. 0.69,

**Table III.** Destruction of skin appendages

| | *Fraction* | *s.d.* | P |
|---|---|---|---|
| Control | 0.73 | 0.171 | |
| Piracetam | 0.64 | 0.098 | >0.05 |
| HBOT | 0.61 | 0.068 | <0.05 |

Piracetam vs. HBOT: $P>0.05$.

**Table IV.** Destruction of epidermal basal layer

| | *Fraction* | *s.d.* | P |
|---|---|---|---|
| Control | 0.87 | 0.081 | |
| Piracetam | 0.78 | 0.068 | <0.01 |
| HBOT | 0.73 | 0.059 | <0.001 |

Piracetam vs. HBOT: $P>0.05$.

**Table V.** (Epi)dermal neutrophil infiltration

| | *Fraction* | *s.d.* | P |
|---|---|---|---|
| Control | 0.69 | 0.104 | |
| Piracetam | 0.60 | 0.127 | >0.05 |
| HBOT | 0.47 | 0.082 | <0.001 |

Piracetam vs. HBOT: $P>0.01$.

$P > 0.05$). For the HBOT group, this difference is highly significant (0.47 vs. 0.69, $P < 0.001$), and the difference is also highly significant between the experimental groups themselves ($P < 0.01$).

## Discussion

### Burn depth

Depth and extension of the burn wound surface are two of the most important determinants of both mortality and morbidity of the burn injury. Although, in recent years, immediate mortality – due to 'burn wound shock' – has decreased significantly, owing to the more efficient fluid resuscitation protocols and the more aggressive surgical approach (early tangential excision), a considerable portion of the burn-related deaths now occurs during the weeks after the insult. These deaths are mostly due to multiorgan failure, respiratory insufficiency and/or systemic infection[6].

As for morbidity, the risk of delayed burn wound healing and hypertrophic scar formation is directly dependent on the depth of the burn wound and hence its chances of re-epithelializing spontaneously.

The longer the duration of the healing process, the higher the risk of wound infection, further compromising proper burn wound healing[7–9].

Apart from the direct cellular death by heat-induced denaturation of proteins, a delayed and progressive necrosis is observed in the zones around the primary burn injury. A number of factors have been associated with this progression of cellular necrosis.

Zawacki[2] has described a progressive capillary stasis in second degree burn wounds, and was able to distinguish two phases:

- 0–4 h: period of oedema formation, progressive capillary stasis.
- 4–24 h: stabilization of oedema and capillary stasis.

By preventing desiccation of the burn in this model, a reversal of capillary stasis could be observed after 24 h, up to the epidermal layers.

In more severe burn wound models, however, as often observed in the clinical situation, these preventive measures, even when associated with 'state of the art' vascular filling and haemodynamic resuscitation, do not succeed in reversing this capillary stasis sufficiently to permit the survival of dermal cells[10].

One of the reasons for this may be found in the sequence of events causing this capillary stasis. During the first hours, the slowing of the capillary blood stream is essentially due to a mechanical compression of the capillaries by oedema formation in the tissues. There, the direct thermal energy overload causes cell lysis and liberation of oncotic substances in the intercellular space, with attraction of fluids from the capillary vascular bed, and elevation of the capillary blood viscosity[11]. This corresponds to the first phase observed by Zawacki[2].

At a certain point, this will induce rouleaux formation of the red blood cells, progressive desaturation of their haemoglobin and progressive hypoxia in the capillaries adjacent to the initial burn injury. The consequences of this hypoxia, such as endothelial cell swelling and initiation/propagation of inflammatory reactions, will increase the permeability of the capillary wall and augment the oedema formation in these zones[12,13].

The causes of oedema formation will thus be progressively shifting from initial extracapillary (increased oncotic pressure and hence increased afterload) to local structural defects (augmented capillary permeability and endothelial cell damage).

A key factor in both the endothelial cell damage and the initiation of the various inflammatory cascades (complement activation, activation of arachidonic acid cycle, coagulation cascade, activation of polymorphonuclear leucocytes) seems to be hypoxia-induced oxygen free radical (OFR) formation[14–17]. In fact, the OFR mediated reactions taking place in the vicinity of the burn wound show striking similarities with those observed in most ischaemia-reperfusion models[18].

In these models, hypoxia induces OFR formation by means of at least two mechanisms: the conversion of xanthine-reductase (X-R) to xanthine-oxidase (X-O)[15,19], and the increase in the natural superoxide radical spill by the reduction of the enzymes of the electron transfer chain in the mitochondria of the endothelial cell[20]. The increased production of superoxide radical initiates an auto-aggravating process, with the leucocytes playing a key role in continuing OFR production[21,22]. This leads to increasing local tissue damage (by the deleterious action of the various OFR species on all cellular membranes), but may also induce distant pathological changes related to (pulmonary, hepatic, renal) migration and subsequent translocation of neutrophils, or to increased production of humoral factors (TNFα, IL6...)[14,16].

Any reduction in the hypoxic capillary and tissue damage is likely to reduce not only the final extent of the local insult, but also the risks of subsequent systemic complications in a burned patient.

### Hyperbaric oxygen therapy and piracetam in the treatment of burns

Since 1965, the possible beneficial influence of HBOT on burn wound healing has been suggested[23]. Because of a lack of randomized prospective clinical trials and financial and/or practical constraints, the therapeutic use of oxygen under pressure has not gained widespread acceptance. There have been, however, a considerable number of experimental reports that document the effects of HBOT when used in the acute phase after the burn injury:

- A decrease in the amount of plasma extravasation (25% vs. 41%) in dogs, submitted to a 40 per cent TBSA third degree burn[24].
- An acceleration and more complete restoration of the capillary permeability (measured by the Chinese Ink infusion technique) in a rat model of 5 per cent TBSA partial-thickness burn[25].
- A preservation of the tissue ATP levels in zones adjacent to the burn wound[26].
- A decrease in oedema formation and exudation rate in and around an (5-mm diameter) experimental partial-thickness burn wound in a human model[27].

These possible effects would be related to:

- A generalized precapillary vasoconstriction, hyperoxia-induced, diminishing the blood flow through the damaged capillaries[28].
- An increase in the quantity of oxygen transported per unit of blood, by physical dissolution of oxygen in plasma (up to 5 ml/100 ml plasma)[29].
- An increase in the intracapillary pressure of oxygen, resulting in an increase of the pericapillary diffusion distance of oxygen[30].
- A possible increase in the plasticity of the red blood cells, diminishing the capillary sludge[31].

A number of clinical reports of the use of HBOT exist that seem to confirm these experimental findings. Notably, a reduction in resuscitation fluid requirements, in the needs for surgical interventions, in duration of hospital stay and in total hospitalization costs, has been noted in patient groups, comparable in age, type of burn and TBSA burned[32]. These ongoing studies, which are more economical, seem to gain importance. They are, however, subject to criticism because of their retrospective nature and a lack of formal randomization.

For piracetam, no animal or human studies are available regarding its use in burns treatment, some being 'en route'. However, the rheological properties of this widely used drug, together with the complete absence of noticeable side-effects, even at extremely high dosages, warranted its formal evaluation for this field of application.

### Experimental set-up

Although the influence of HBOT as a therapeutic measure 'on its own' has been demonstrated in the experimental setting, few animal studies have been performed to evaluate the supplemental benefit of HBOT to a classical burn wound treatment protocol, in preventing the extension of the burn injury.

Therefore, our protocol was designed to resemble a 'realistic' burn patient treatment scenario:

- A delay of 3–4 h before initiation of advanced burn wound management.
- Daily wound dressing changes, with application of an antimicrobial agent.
- Utilization of a HBOT protocol that is currently accepted and employed in the ancillary treatment of burned patients[33].
- Utilization of a currently recommended high dosage of piracetam (200 mg/kg b.i.d. IM).

As an antimicrobial agent for this study we have chosen mafenide hydrochloride. Although silver sulfadiazine 1 per cent cream is more commonly used in burn centres throughout the world, its greasy component is likely to be incompatible with external high pressure oxygen exposure (risk of explosion), and may represent a serious fire risk when treating these patients in a monoplace hyperbaric chamber.

Mafenide, although not commercially available as an aqueous solution, can be obtained in powder form, and in our burn centre it is commonly used in a 10 per cent solution, as in the commercially available cream. This unusual pharmacological presentation does not, to our view, affect the validity of the results of our study.

For this study, no haematological or serological parameters have been studied, for the burn wound inflicted was small (5 per cent TBSA) and would not routinely need important intravenous fluid resuscitation. Also, the risk of distant complications is, in this type of injury, virtually non-existent.

Lung tissue biopsies, taken from animals of preliminary groups after completion of the study period, showed no gross pathological alterations (data not shown) in either group. Any serological alterations would be likely to be either unmeasurable or of no clinical significance in this burn wound model.

### Results

HBOT and piracetam were both able to decrease the amount of (epi)dermal cellular destruction, as well as the degree of inflammatory reaction (dermal leucocyte infiltration), when applied early after the burn infliction. This could represent an added benefit when compared to a 'classical' burn wound treatment only.

Some reservations have to be made, however, as to the interpretation of these results:

1. It is not known how big a fraction of skin appendages needs to be preserved to ensure spontaneous burn wound healing. The difference in appendage destruction is small but (for the HBOT group) statistically significant. Whether this will result in faster healing of the burn wound is impossible to appreciate from this study.
2. The same remark can be made for the percentage of destruction of the surface epithelium. This destruction is, obviously, mainly induced by the direct thermal energy directed to these cells. The fact that HBOT or piracetam, either via an increased availability of oxygen or by some other mechanism, can preserve a bigger portion of these cells, is interesting, but here again, the difference is very small, and the clinical importance is questionable.

The decrease in polymorphonuclear leucocyte infiltration in the HBOT group is the most striking observation. Being admittedly only an imprecise indicator of the extent of the inflammatory reactions that are taking place, neutrophil adherence to the capillary wall, followed by rolling and translocation, is one of the earliest and most easily observable signs of their activation[17,22,34,35]. It seems that HBOT is able to significantly reduce this leucocyte migration.

## Conclusions

This study addressed, from a morphological, descriptive point of view, the possible benefits of HBOT and piracetam when used in conjunction with conventional burn wound treatment. No information was obtained as to the final clinical consequences of these ancillary treatments, because animals were killed before wound healing. However, the effects of HBOT, notably on the degree of inflammatory leucocyte response, are important, and warrant further investigation.

A biochemical study, producing a more significant burn injury, and subsequent serological measure-

ments, as well of their effects on OFR production, will therefore be undertaken.

This study suggests that HBOT may have a place in the combined early management of the burn injury.

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## References

1 Jackson DM. The diagnosis of the depth of burning. *Br J Surg* 1953; **40:** 588.
2 Zawacki BE. The natural history of reversible burn injury. *Surg Gynecol Obstet* 1974; **139:** 867.
3 Boykin JV, Eriksson E, Pittman N. In vivo microcirculation of a scald burn and the progression of postburn dermal ischaemia. *Plast Reconstr Surg* 1980; **81:** 741.
4 Mathieu D, Wattel F, Pellerin PH. Oxygénothérapie hyperbare et chirurgie réparatrice. In: Wattel F, Mathieu D (eds) *Oxygénothérapie et réanimation.* Paris: Masson, 1990; p 192.
5 Kaufman T, Lusthaus SN, Sagher U et al. Deep partial skin thickness burns: a reproducible animal model to study burn wound healing. *Burns* 1990; **16:** 13.
6 Warden GD. Burn shock resuscitation. *World J Surg* 1992; **16:** 16.
7 Deitch EA, Wheelahan TM, Paige RM et al. Hypertrophic burn scars: analysis of variables. *J Trauma* 1983; **23:** 895.
8 McDonald WS, Deitch EA. Hypertrophic skin grafts in burned patients: a prospective analysis of variables. *J Trauma* 1987; **27:** 147.
9 Robson MC, Barnett RA, Leitch IOW et al. Prevention and treatment of postburn scars and contractures. *World J Surg* 1992; **16:** 87.
10 Heimbach D, Engrav L, Grube B et al. Burn depth: a review. *World J Surg* 1992; **16:** 10.
11 Arturson G, Mellander S. Acute changes in capillary filtration and diffusion in experimental burn injury. *Acta Physiol Scand* 1964; **62:** 457.
12 Ganrot K, Jacobson S, Rothman V. Transcapillary passage of plasma proteins in experimental burns. *Acta Physiol Scand* 1974; **91:** 297.
13 Lund T, Onarheim H, Reed RK. Pathogenesis of edema formation in burn injuries. *World J Surg* 1992; **16:** 2.
14 Till GO, Hatherill JR, Tourtelotte WW et al. Lipid peroxidation and acute lung injury after thermal trauma to skin. Evidence of a role of hydroxyl radical. *Am J Pathol* 1985; **119:** 376.
15 Woolliscroft JO, Prasad JK, Thomson P et al. Metabolic alterations in burn patients: detection of adenosine triphosphate degradation products and lipid peroxides. *Burns* 1990; **16:** 92.
16 Youn YK, Lalonde C, Demling R. The role of mediators in the response to thermal injury. *World J Surg* 1992; **16:** 30.
17 Ward PA, Till GO. The autodestructive consequences of thermal injury. *J Burn Care Rehabil* 1985; **6:** 251.
18 Traystman RJ, Kirsch JR, Koehler RC. Oxygen radical mechanisms of brain injury following ischemia and reperfusion. *J Appl Physiol* 1991; **71:** 1185.
19 McCord JM. Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med* 1985; **312:** 159.
20 Flaherty JT, Zweier JL. Role of oxygen radicals in myocardial reperfusion injury: experimental and clinical evidence. *Klin Wochenschr* 1991; **69:** 1061.
21 Ward PA, Mulligan MS. New insights into mechanisms of oxyradical and neutrophil mediated lung injury. *Klin Wochenschr* 1991; **69:** 1009.
22 D'Alesandro MM, Gruber DF. Quantitative and functional alterations of peripheral blood neutrophils after 10% and 30% thermal injury. *J Burn Care Rehabil* 1990; **11:** 295.
23 Wada J, Ikeda T, Kamata K et al. Oxygen hyperbaric treatment for carbon monoxide poisoning and severe burns in coal mine gas explosion. *Igakunoayumi (Japan)* 1965; **54:** 68.
24 Wells CH, Hilton JG. Effects of hyperbaric oxygen on postburn plasma extravasation. In: Davis J, Hunt TK (eds) *Hyperbaric Oxygen Therapy.* Bethesda, MD: Undersea and Hyperbaric Medical Society 1977; p 259.
25 Miller TA, Korn HN. Epithelial burn injury and repair. *Arch Surg* 1977; **112:** 732.
26 Stewart RJ, Yamaguchi KT, Cianci PA et al. Effects of hyperbaric oxygen on adenosine triphosphate in thermally injured skin. *Surg Forum* 1988; **39:** 87.
27 Hammarlund C, Svedman C, Svedman P. Hyperbaric oxygen treatment of healthy volunteers with u.v.-irradiated blister wounds. *Burns* 1991; **17:** 296.
28 Eggers GW, Paley HW, Leonard JJ et al. Hemodynamic responses to oxygen breathing in man. *J Appl Physiol* 1962; **17:** 75.
29 Bauer P, Larcan A, Broussole B. Effets cardio-respiratoires de l'oxygène hyperbare. In: Wattel F, Mathieu D (eds) *Oxygénothérapie hyperbare et réanimation.* Paris: Masson, 1990; p 11.
30 Sheffield PJ. Tissue oxygen measurements. In: Davis JC, Hunt TK (eds) *Problem Wounds. The role of oxygen.* London: Elsevier, 1988; p 37.
31 Mathieu D, Coget J, Saulnier F et al. Filtrabilité érythrocytaire et oxygénothérapie hyperbare. *Méd Sub Hyp* 1984; **3:** 100.
32 Cianci P, Lueders HW, Lee H et al. Adjunctive hyperbaric oxygen therapy reduces length of hospitalisation in thermal burns. *J Burn Care Rehabil* 1989; **10:** 432.
33 Hart GB, Grossman AR. Thermal burns. In: Kindwall EP, Goldmann RW (eds) *Hyperbaric Medicine Procedures.* Milwaukee: St Luke's Medical Center 1988; p 98.
34 Zamboni WA, Roth AC, Russell RC et al. Morphological analysis of the microcirculation during reperfusion of ischaemic skeletal muscle and the effect of hyperbaric oxygen. *Plast Reconstr Surg* 1993; **91:** 1110.
35 Arturson G. The pathophysiology of severe thermal injury. *J Burn Care Rehabil* 1985; **6:** 129.

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