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Perfluorochemicals as a treatment of decompression sickness in rats

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Abstract. Perfluorodecalin and perfluorotripropylamine which have N_2 solubility coefficients of 28.4 and 35.7 ml/dl, respectively, were used for treatment of decompression sickness in this study. Rats with chronically implanted venous catheters were held for 30 min at 800 kPa (7 bar, 8 ATA) by introducing compressed air into a chamber in which they were kept; a relatively short period of decompression followed (200 kPa/min). Immediately thereafter injections of the perfluorochemicals (PFCs) in a dose of 10 g/kg were given, controls received saline in the same volume or remained without treatment. An observation period of 2 h followed; after this time the incidence of death amongst the experimental animals (as compared with controls tested by the χ^2-test) showed that PFC treatment increased the likelihood of survival. Probit-log time relationship for the incidence of death also revealed a significant decrease in lethality in treated rats 30 min after the end of decompression. The mean lethal times Lt_{50} differed significantly, too.

A still greater effect might be expected if the PFC emulsion were deprived of its normal nitrogen content by oxygenation before administration. Under the conditions of the present experiments PFCs produced an improvement in N_2 exhalation at least in terms of the survival rate after compression followed by a very short decompression time.

Key words: N_2 solubility – Hyperbaria – Asymmetric compression – Decompression

Introduction

Perfluorochemicals (PFCs) have proved to be valuable carriers for the respiratory gases O_2 and CO_2. The solubility factors of these gases in PFCs which are most widely used are respectively 20 and 3 times that in plasma (Table 1). In practice the solubilities are not as high as this because efficient emulsions seldom contain more than 20% of the PFCs. However, the improvement of gas transport is caused not only by a higher solubility, but also by the small particle size of the emulsion (weighted average diameter 0.12 μm). Thus a 20% (weight/volume) PFC emulsion offers a surface area about 100 times that of the red cells in blood with a hematocrit of 45% [12].

The solubility of a third respiration gas, N_2, is also more than 20 times higher in PFCs than in plasma (Table 1) and approximately 5-fold greater in a 20% emulsion than in plasma. Thus experiments were performed to test wether the higher N_2 solubility of PFCs could be used in the treatment of decompression sickness in rats.

Materials and methods

Male Wistar rats with 220–320 g body weight (bw) were used throughout these studies. They were housed in groups of four in macrolon cages, kept on standard diet (Altromin, Lage, FRG) and had water ad libitum. Under pentobarbital anesthesia (30 mg/kg bw) the right external jugular vein was cannulated with a heparin-filled portex tube (0.8 mm external diameter). The free end was drawn through the skin of the neck and closed with a short steel needle. After surgery the animals received, through the catheter, a daily dose of ampicillin, 30 mg/kg and oxycillin, 20 mg/kg (Totocillin) dissolved in 0.1 ml/kg of heparinized saline. Twentysix animals with patent catheters and healthy in appearance were randomly divided into an experimental group which would be treated with PFC and a control group. Some of the control rats received a saline injection after decompression, the others got no treatment. The experimental group received 10 g PFC/kg bw in the form of Fluosol DA (The Green Cross Corp., Osaka, Japan), containing a mixture of 7 parts perfluorodecalin and 3 parts perfluorotripropylamine emulsified with 3.4% pluronic F 68, 1% glycerol and 0.5% yolk phospholipids. This dose produced an initial intravascular concentration of approximately 5.9 ml PFCs/dl blood, the intravascular half-life being more than 10 h [10].

For the compression phase of the experiments the rats were placed in groups of four in a small tank provided with a petri dish containing soda lime (Dräger-sorb 650, Dräger, Lübeck, FRG) as a CO_2 absorbent. The tank was closed

Table 1. Solubility coefficients (ml/dl per atm at 37° C) for the used PFCs and plasma

Compound	Gas		
	O_2	CO_2	N_2
FDC	45.4[a]	153[b]	28.4[a]
FTPA	45.5[a]	166[a]	35.7[a]
Plasma	2.14[c]	51.3[c]	1.17[c]

[a] Yokoyama [20]
[b] Cottrell [4]
[c] Bartels [1]

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with a 20 mm thick, steel-armoured acrylic glass plate which allowed observation of the animals. The temperature was held at 24 ± 2° C by means of a heating bulb. Pressurization was produced with compressed air delivered from a cylinder to give an end pressure in the tank of 800 kPa (8 atmospheres absolute pressure, 8 ATA).

The time course of the compression procedure is shown in Fig. 1. It began with a slow initial phase of 100 kPa/30 s. The pressure was then doubled every 30 s. Thus according to Boyle's law, compression of a given volume of air in the enclosed compartments per unit time remained constant. Using this method the pressure increase appeared to be well tolerated by the animals without signs of unusual behavior or evidence of pain from the middle ear. The time spent at full hyperbaria, "bottom time", amounted to 30 min. During the decompression phase the pressure decreased in a linear fashion at 200 kPa/min. Thus it lasted for 3.5 min. The

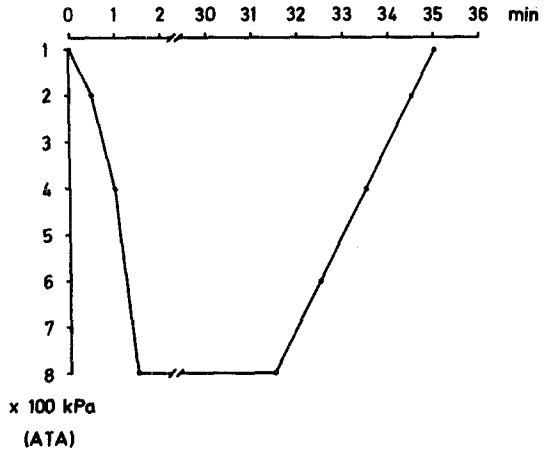

Fig. 1. The pressurization profile used, with asymmetric compression and decompression times. Compression was adapted to Boyle's law with the same relative pressure increase occurring in equal time-periods

i.v. injections were given immediately after decompression. Then the animals were put into cages and a 2 h period of observation followed. Later, observation of the animals was carried out at longer time intervals. Times of death for up to 12 h after decompression were taken into consideration, but not after this time, for death in animals which had returned to normal function after decompression could have been secondary to infections or to complications resulting from the implanted catheters.

Statistical approach

The ratio of survival. Death at 2 h after the end of decompression was first established and tested with the χ^2-test. In addition, the incidence of death was transformed to probits [6] and plotted against the logarithm of time, and the respective regression lines were calculated. The natural logarithm (base e) was preferred to the decadic one as giving a more suitable scale for calculations and the graph. Comparison of the regression lines was performed by comparing the differences between the slope constant b and by comparing several points on both lines, using regression analysis [5, 17] and the method of Zerbe et al. [22]. To calculate the mean lethal time Lt_{50} (the time at which 50% of the animals were dead) the probits of 5 were used in the reversed function of $xf(y)$ together with their standard deviation for individual observation. Finally the logarithmic values were recalculated as time values and expressed in minutes and hours.

Results

At 2 h after the end of decompression the χ^2-test revealed a significant improvement of the survival rate for the PFC treated animals ($\chi^2 = 4.46$; $P<0.05$), while no difference could be shown between saline infused rats and untreated controls.

The relationship between the probit-transformed lethality and the natural logarithm of the time after the end of decompression is plotted in Fig. 2. (The expression "lethal-

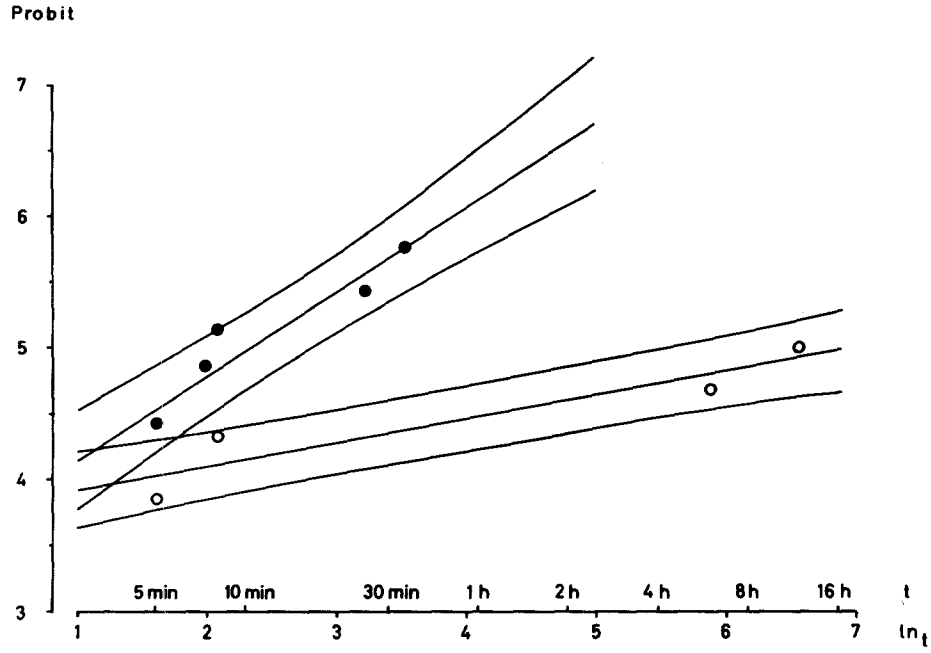

Fig. 2
Probit (of lethality) related to the ln of time after the end of decompression. Besides the regression lines the limits of the standard deviations for individual observations are drawn. *Black circles:* controls, *open circles:* PFC treated rats

Table 2. Differences in probits of lethality for different times after decompression ($n = 8-9$ rats in each group

		Time	
		6 min	30 min
Control	probit	4.658	5.669
PFC	probit	4.045	4.351
Δ	probit	0.613	1.318
	s Δ	± 0.234	± 0.220
	P^a	n.s.	<0.01

[a] Considering the Scheffé criterion $t \geq \sqrt{2F\alpha}$

Table 3. Comparison of mean lethal times Lt_{50} ($n = 8-9$ rats in each group)

	$\ln Lt_{50}$	Lt_{50}
Controls	2.366 $s\hat{y} \pm 0.426$	10.66 min
PFC rats	6.620 $s\hat{y} \pm 1.479$	12.51 h
P	<0.05	

ity" was preferred to "mortality" since according to epidemiological definition [19], mortality describes the number of death of a whole population while lethality is the number of death in an affected population.)

The two regression lines diverge strongly; their equations are

$y_{\text{contr.}} = 0.634\,x + 3.513$ $\quad sb_{y,x} = \pm 0.159$ and
$y_{\text{PFC}} = 0.179\,x + 3.74$ $\quad sb_{y,x} = \pm 0.050$.

The slopes b differ significantly ($P<0.05$), that of the controls being distinctly greater.

A comparison of the two regression lines at several values of x revealed the following points. At 6 min after the end of the decompression time the difference between the lines was not yet statistically significant. However, 30 min after decompression there was a highly significant difference (Table 2). At this time the mean values for lethality, expressed as a percentage, amounted to 74.8% and 24.8%, respectively. A calculation of Lt_{50} for the two groups gave values of 10.7 min and 12.5 h as shown in Table 3.

The number of animals which showed signs of paralysis or delayed shock symptoms and survived was negligible. In nearly all cases, limping or hemiplegia was followed by asphyxial death, convulsions appeared only in the first 10 min and were followed by immediate death. The surviving animals showed no characteristic signs of "bends".

Discussion

It is appropriate first to compare the methodological approach adopted in the present experiments with those used in other published studies. Increases in chamber pressure of 75 kPa/min [18], 180 kPa/min [2, 15, 16], 1000 kPa/min [14] and even 6000 kPa/min [7] have been employed by others, and even the most rapid compression appeared to be easily tolerated by rats according to Flynn and Lambertsen [7]. However, other authors have reported that some signs of anxiety or discomfort do occur [18] which may have been due to a lag in the pressurization of the middle ear. The method we have adopted of doubling of the pressure in equal time periods beginning with 100 kPa/30 s seemed to be a logical and physiological procedure which would avoid this problem whilst also economizing on the time used for the whole experiment.

The time spent at the maximal pressure of up to 1500 kPa (15 ATA) was 15 min in experiments on mice and this was presumed to allow sufficient time for full saturation with nitrogen [2, 7]. For rabbits under a pressure of 1000 kPa (10 ATA), 50 min were chosen [13]. Thus the 30 min period used in the present experiments seems to be comparable with previous studies, though there is the possibility that it may have failed to give a full saturation in rats. Even if this is the case this causes no limitation of the results, for most human divers below 100 m sea level do not reach saturation.

The velocity of decompression for divers generally amounts to 18 m/min (60 feet/min) which equals 180 kPa/min. Other authors have used a decompression period lasting only a few seconds to look for special symptoms [2, 7, 15]. In the present study, in order to obtain good survey of a manually controlled pressure system which had to be adjusted in accordance with a manometer calibrated in bar, we chose a decompression rate of 200 kPa/min (2.0 bar/min).

The dose of PFC administered in the present experiments was in the middle of the range used in other animal experiments [3, 11, 21]. This dose in a 300 g rat could have taken up 0.5 ml nitrogen per atmosphere pressure from the tissues into the circulation (3 g of the emulsion contains 1.64 ml pure PFCs and could dissolve 0.5 ml N_2 per atmosphere at 37° C). The whole-animal tissue content of nitrogen in a 300 g rat at 800 kPa exceeds that under normal atmospheric conditions by about 20 ml N_2, and so only a small part of that N_2 load could be taken up by PFCs during one circulation time. This being the case it is obvious that improved transport function rather than the simple absorption of the tissue nitrogen load must have been responsible for the beneficial effect. Certainly the large surface area of the PFC particles could have made a significant contribution. A further increase in the dose would not seem to be advisable because there are some side effects of PFCs which are strongly dose dependent and secondary to storage in liver, spleen and the other organs of the reticulo-endothelial system [9, 11, 21].

An even better effect might be expected if the PFCs were to be deprived of their content of atmospheric nitrogen before being administered. This could be achieved by initial treatment of the emulsion in an oxygenator so as to allow equilibration with pure oxygen. In the present studies only a part of the nitrogen diffusing from desaturating tissues could be taken up and transported to the lung. An additional amount of nearly 0.5 ml nitrogen per 300 g rat could have been dissolved if the emulsion had been gas exchanged. This 0.5 ml might be enough to threaten the life of some animals.

Although we took steps to absorb CO_2 in the tank, no attempt was made to reduce the increase in the partial pressure of oxygen. Thus the possibility of O_2 toxicity must be kept in mind. At 800 kPa (8 ATA) the partial pressure of O_2 reaches 168 kPa (1.68 ATA). However, this value for a time of 30 min employed in the present experiments is below the safety limit for respiration of hyperbaric oxygen at dives

which is 175 kPa (1.75 ATA) during 75 min, according to Lanphier [8].

Administration of a volume of saline equal to the volume in which PFC was delivered had no positive effect of survival, even though a better fluidity of blood might have been expected to improve N_2 transport. This result, though, was similar to that obtained by Reeves and Workman [16], for administration of different doses of heparin proved to be ineffective as treatment for decompression sickness. Similarly many drugs, hypertonic solutions, plasma expanders, lipemia-clearing agents and anti-coagulants have come under scrutiny in the search for a treatment of decompression sickness [16], but none have proved successfull. On the basis of the present study it seems that a step towards the development of a suitable treatment could be made by further experiments on the use of PFCs.

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