Clinical Hemorheology and Microcirculation 19 (1998) 49–81
IOS Press

First International Conference on Microcirculation in Hypertension*

Hypertension, hemorheology and oxygen transport, Bari, Italy, 1996

Guest editors: M.R. Hardeman** and A.J. van der Kleij

General introduction

Prof. A. Pirrelli

The aim of this meeting is to evaluate of the correlation between hemorheology and arterial hypertension. This is an old hypothesis, but it has not been well demonstrated. I think that the conditions causing impairment of flow in small and big arteries are also important factors in arousing hypertension and vascular damage.

Pappenheimer (1942, 1958), Bayliss (1962) and others concluded from their studies on some pathological conditions that variations of hematocrit induced hemodynamic changes in blood circulation and that this could be attributed to an increase in blood viscosity. Later, Dintenfass (1981) supported this concept and demonstrated that increased viscosity and cell rigidity can play an important role in hypertensive disease. He also suggested the presence of viscoreceptors on arterial walls, which may be very sensitive to viscosity changes and could include an autoregulatory hemodynamic mechanism. This mechanism may be primary at the beginning of essential hypertension or secondary in stabilised hypertension.

Papers on increased viscosity during hypertensive disease are scarce; this may probably be attributed to very difficult and expensive methodology for the measurement of blood viscosity and its determinants.

At present a new instrument, measuring red cell deformability and aggregation, based on the physical principles of laser diffraction and backscatter, respectively, has become available. Furthermore, polarographic techniques are available to evaluate transcutaneous PO_2 for assessment of O_2 transport in small peripheral arteries.

We are using some of these techniques, but we would also like to know more about them and compare them. For these reasons I decided to organise this meeting; to compare and discuss the results of experiments.

*Under the auspices of Dimo-Sezione Medicina Interna ed Ipertensione, Universita di Bari, Italy, and the Department of Surgery and Clinical Hemorheology, Academic Medical Center, University of Amsterdam, The Netherlands.

**Corresponding author: M.R. Hardeman Ph.D., Department of Internal Medicine, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. Fax: +31 20 5664440; E-mail: M.R.Hardeman@amc.uva.nl.

Clinical hemorheology placed in perspective to hypertension: Introduction and implementation of new methodology

M.R. Hardeman Ph.D.
Department of Internal Medicine, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

1. Introductory remarks

1.1. Hemorheology

Viscosity is defined as the internal resistance to flow; sometimes the term fluidity is used with the opposite meaning, i.e., the capacity to flow.

When we consider a fluid as composed of a parallel layer, viscosity is also a measure for the friction of two adjacent layers moving relative to each other. It is clear that in the centre of a tube the friction is less and the layers move faster than those located on the stationary wall, resulting in a parabolic flow profile.

Thus, the velocity of a layer relative to the next layer (called shear rate) is directly proportional to the force per unit area exerted on that layer (called shear stress) and reciprocally proportional to the internal friction or viscosity.

Viscosity is, however, only one determinant of the peripheral resistance, geometrical factors as vessel length and diameter also play a role. Poiseuille has combined these viscous and vascular factors, resulting in the famous equation named after him:

$$Q = \Delta P \frac{\pi r^4}{8 l \eta};$$

$Q =$ flow, perfusion rate; $\Delta P =$ mean pressure difference; $\eta =$ viscosity; $l =$ vessel length; $r =$ vessel radius.

On closer examination of this relation, it is clear that the vessel diameter (or the radius), present in the fourth power, is the dominating factor determining flow, in contrast to the single (reciprocal) power of viscosity. We have to realise therefore, that the effect of small changes in viscosity can easily be abolished by vasomotoric adaptations. Therefore, measurement of the overall property of blood viscosity can be expected to be of significance only in those situations where the vasomotoric capacity is impaired but also in the situation of a sustained vasoconstriction.

With respect to their viscous behaviour, most fluids are Newtonian, i.e., their viscosity is a constant property, only dependent on temperature. An example in our daily life of such a Newtonian fluid is motor oil. At a fixed temperature the viscosity is constant at all shear rates, and the only thing we had to do in wintertime (before the introduction of the multigrade quality!) was change the oil for another one with lower viscosity.

There are, however, also fluids whose viscosity behaviour is non-Newtonian, in other words, their viscosity is not a constant characteristic but shear rate dependent. An example in our daily life is tomato ketchup.

A certain resistance has to be overcome before it starts to flow. The force needed to accomplish this is called yield stress. Furthermore, as soon as non-Newtonian fluids start to flow, the apparent viscosity decreases and as a result of this, flow increases, etc. We call this behaviour shear thinning.

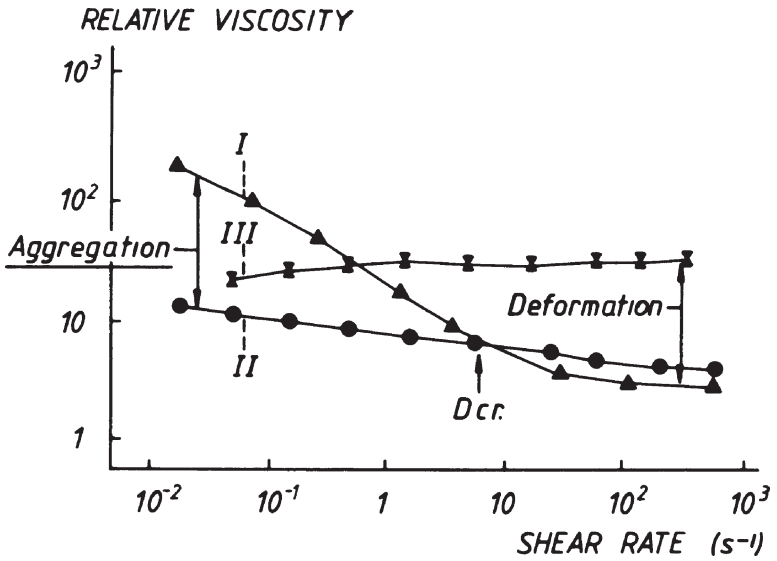

Fig. 1. Relative viscosity (cP) plotted versus shear rate (s^{-1}) for: (I) whole blood, (II) RBC suspended in saline, (III) rigidified RBC resuspended in plasma. From Chien [1].

Dealing with blood we also notice non-Newtonian behaviour: after the yield stress has been overcome, there is an exponential decrease in viscosity at increasing shear rates.

A very simple, almost classical experiment, described by Chien [1], can shed some light on the mechanism behind this non-Newtonian character of blood viscosity (Fig. 1). Curve I shows the exponential increase in viscosity at decreasing shear rates of whole blood. Then, this blood is centrifuged, the supernatant plasma removed and exchanged for the same volume of saline after which the cells are resuspended. Viscosity measurements are repeated now with this RBC–saline mixture having the same hematocrit as blood. This results in curve II. As can easily be checked by microscopy, the lower viscosity at low shear rates compared to normal blood is caused by the absence of RBC aggregation (or rouleau formation). Some plasma proteins, especially fibrinogen, are needed for RBC aggregation. Now, let us centrifuge the suspension again, separate the supernatant and treat the RBC with glutaraldehyde, a protein crosslinking agent. After the rigidified cells are washed, they are resuspended in their plasma and viscosity is measured again as a function of shear rate. This results in curve III, which shows a significantly higher viscosity than normal blood at high shear rates. Apparently, the capacity of normal RBC to deform is very important to keep the viscosity at high shear rates low. Despite the presence of plasma proteins, such rigid RBC do not aggregate or form loose aggregates. The experimental results, cited above, hold for the macrocirculation. In the smallest capillaries of the microcirculation, however, the geometric constraint is the most saillant parameter making the cell's capacity to deform a conditio sine qua non for an undisturbed microcirculation.

Besides the quality of the individual RBC to deform and to aggregate with other RBCs, the volume they take relative to whole blood, the hematocrit, is a dominating factor determining blood viscosity. Since the total mass of RBC present is directly proportional to the amount of oxygen that can be bound, it is the transport capacity which will limit oxygen availability to the tissues at high hematocrit and viscosity, in other words, there is an optimal hematocrit. This value is lower than the mean normal hematocrit.

1.2. Hypertension

A more clinical way of expressing the above-cited Poiseuille equation

$$\mathrm{MAP} = \mathrm{CO} \times \mathrm{R},$$

encompassing mean arterial pressure (MAP), cardiac output (CO) and the resistance to flow (R). Since both vascular hindrance (vasoconstriction) and blood viscosity contribute to R it is, at least on theoretical grounds, clear that an elevation of arterial pressure must be analysed, not only in terms of cardiac output and/or arteriolar constriction, but also in terms of blood viscosity.

Essential hypertension is characterised by sustained diastolic blood pressure in excess of 95 mm Hg and by the fact that no cause for the disorder has been found. Essential hypertension is heterogeneous in its hormonal and biochemical patterns. Cardiac output is usually normal. The sustained elevated blood pressure is mainly caused by an increase in total peripheral resistance, which in its turn is associated with both an excessive vasoconstriction of the arteriolar smooth muscle [2] and with an increased blood viscosity [3]. The latter may result in structural changes in arterioles [4].

The kidney plays a central role both in the cause and in the effect of hypertension. Some of the pathophysiological characteristics of essential hypertension are: altered renal physiology with accelerated natriuresis and reduced renal blood flow. The latter has been associated with renal vasoconstriction and increased glomerular filtration fraction.

The earliest convincing abnormality (i.e., reduction) in regional flow appears to occur in the kidneys [5–7]. Whether they precede or follow the onset of hypertension itself has not yet been determined. It was established, however, that the kidney's Na^+-excreting capacity is highly sensitive to slight changes in renal blood flow [8]. The slightest reduction of renal blood flow leads to a relatively greater fall in renal Na^+ and water excretion. This, in its turn, results in renin secretion, which stimulates angiotensin II production followed by constriction of the arterioles and increase of blood pressure. It is at this level where abnormal bloodviscosity (determinants) might have a causative role for hypertension.

Despite the crucial role for the kidney, the heart is frequently more affected in hypertensive disease. Cardiac hypertrophy in adults is usually caused by hypertension. The development of left ventricular hypertrophy is directly related to whole blood viscosity [9].

Many authors were able to establish a significant positive correlation between arterial pressure and (a determinant of) blood viscosity (reviewed in [4]). Letscher [10] came closest in answering the cause, consequence or coincidence question by demonstrating an increased blood viscosity in the early phase of borderline elevation of arterial pressure. A positive correlation between the severity of essential hypertension and the degree of increased blood viscosity or its determinant RBC aggregation has been demonstrated by Petralito [11].

However, even if increased blood viscosity (or a change in one or more of its determinants) appears to be only an epi-phenomenon accompanying essential hypertension, it is still a risk factor for the development of cardiovascular complications and this should therefore be watched closely and, if possible, controlled.

In vitro measured whole blood viscosity is, in contrast to plasma viscosity, difficult to interpret in clinical terms. Several factors that determine viscosity, such as hematocrit and shear rate, vary considerably in the macro- and microcirculation or are absent *in vitro* (like the Fåhraeus–Lindquist phenomenon and vascular wall). It might be more relevant therefore, to analyse whether and to what extent important blood viscosity determinants (such as plasma viscosity, hematocrit, RBC deformability and RBC aggregation) deviate from normal.

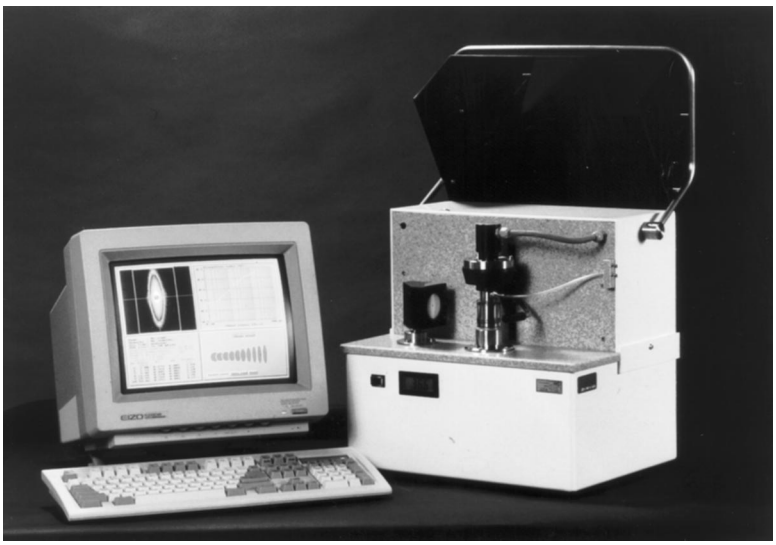

Fig. 2. Laser-assisted Optical Rotational Cell Analyser (LORCA).

1.3. Instrumentation

A new instrument called LORCA (Mechatronics, Hoorn, The Netherlands) has been developed for automated measurement of both red cell deformability and red cell aggregation kinetics (Fig. 2). Details are described elsewhere [12–14]. In short:

- *Red cell deformability* is measured from the diffraction pattern obtained with a laser beam traversing a suspension of red cells in a high viscous medium. Resting cells produce a circular diffraction pattern, while cells which are submitted to shear stress elongate, resulting in a ellipsoid pattern. An ellipse-fit computer program can calculate the elongation index (EI).
- *Red cell aggregation* is measured with the same instrument, now using the backscatter signal from whole blood. Blood is sheared and while all cells are dispersed, the motor is stopped suddenly, causing cell aggregation. The change in intensity of backscattered light is measured and computer assisted analysis of the aggregation curve yields indices of the extent of aggregation, the kinetics of the process, as well as the minimal force needed to prevent aggregation.

Further possible features of the LORCA are:

- *red cell (membrane) relaxation rate*, which can be estimated according to Baskurt et al. [15];
- *red cell internal viscosity*, which can be estimated through a “viscoscan” [16].

2. Conclusions

1. There is at least a strong association between the occurrence of essential hypertension and increased blood viscosity (determinant).

2. Up until now, direct proof of a causative role of an increased determinant of blood viscosity in the onset of hypertension is lacking.

3. Further studies into this matter are warranted since we now have at our disposal an instrument suitable for measurement of multiple structural hemorheological red cell parameters, which may directly be involved in the pathogenesis of hypertension.

References

[1] S. Chien, Present state of blood rheology, in: *Hemodilution, Theoretical Basis and Clinical Application*, E. Messmer and H. Schmid-Schönbein, eds, Karger, Basel, 1972, pp. 1–45.
[2] B. Folkow, Physiological aspects of primary hypertension, *Physiol. Rev.* **62** (1982), 347–503.
[3] B. Sandhagen, G. Frithz, W. Waern and G. Ronquist, Increased whole blood viscosity combined with decreased erythrocyte fluidity in untreated patients with essential hypertension, *J. Intern. Med.* **228** (1990), 623–626.
[4] A. Chabanel and S. Chien, Blood viscosity as a factor in human hypertension, in: *Hypertension: Pathophysiology, Diagnosis and Management*, J.H. Laragh and B.M. Brenner, eds, Raven Press, New York, 1990, pp. 329–337.
[5] S.C. Sommers, Hypertension and kidney disease, *Prog. Cardiovasc. Dis.* **8** (1965), 210.
[6] N.R. Hollenberg, D.F. Adams and H. Soloman, Renal vascular tone in essential and secondary hypertension: hemodynamic and angiographic responses to vasodilators, *Medicine* (*Baltimore*) **54** (1975), 29–44.
[7] P.W. de Leeuw, T.L. Kho and H.E. Falke, Hemodynamic and endocrinological profile of essential hypertension, *Acta Med. Scand.* **622**(Suppl.) (1978), 9–85.
[8] C.B. Mueller, A. Surtshin, M.R. Carlin and H.L. White, Glomerular and tubular influences on sodium and water excretion, *Am. J. Physiol.* **165** (1951), 411.
[9] R.B. Devereux, J.L. Drayer and S. Chien, Whole blood viscosity is a determinant of cardiac hypertrophy in systemic hypertension, *Am. J. Cardiol.* **54** (1984), 592.
[10] R.L. Letscher, S. Chien and T. Pickering, Elevated blood viscosity in patients with borderline essential hypertension, *Hypertension* **5** (1983), 757–762.
[11] A. Petralito, Erythrocyte aggregation in different stages of arterial hypertension, *Thromb. Haemost*. **54** (1985), 555.
[12] M.R. Hardeman, P.T. Goedhart, J.G.G. Dobbe and K.P. Lettinga, Laser-assisted Optical Rotational Cell Analyser (LORCA); I. A new instrument for measurement of various structural hemorheological parameters, *Clin. Hemorheol.* **14** (1994), 605–618.
[13] M.R. Hardeman, P.T. Goedhart and N.H. Schut, Laser-assisted Optical Rotational Cell Analyser (LORCA); II. Red blood cell deformability; elongation index versus cell transit time, *Clin. Hemorheol.* **14** (1994), 619–630.
[14] M.R. Hardeman and P.T. Goedhart, Laser-assisted Optical Rotational Cell Analyser (LORCA); Red blood cell aggregometry, in: *Haemorheology & Erythrocyte Aggregation*, Vol. 4, J.F. Stoltz, ed., Editions Médicales Internationales, Paris, 1994.
[15] O.K. Baskurt and H.J. Meiselman, Determination of red blood cell shape recovery time constant in a couette-system by the analysis of light reflectance and ektacytometry, *Biorheology* **33** (1996), 489–504.
[16] M.R. Hardeman and P.T. Goedhart, Laser-assisted Optical Rotational Cell Analyser (LORCA); "Viscoscan" of RBC deformability and estimation of RBC internal viscosity, *Biorheology* **32** (1995), 357.

Which method can be used to evaluate tissue oxygenation?

A.J. van der Kleij M.D. Ph.D.
Department of Surgery (Hyperbaric Medicine), Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

1. Introduction

One major aim of the macro- and microcirculation is to carry nutrients and energisers (oxygen) to the cells, and to take away the end products of cell metabolism. Since there is a very limited capacity for oxygen storage in the cell and interstitial fluids, a constant oxygen supply is essential for normal cellular function.

A large number of factors such as inter-capillary distance, capillary radius, capillary flow, capillary oxygen content, red blood cell size, adherence of cellular blood components, blood viscosity and oxygen diffusion coefficient are involved to regulate the available amount of molecular oxygen for the cell.

2. The microcirculation

Oxygen tension adjacent to cells is influenced by the utilisation of oxygen within that cell. High oxygen consumption in the cell reduces oxygen concentration outside the cell. The steep concentration gradient will accelerate diffusion (Silver [31]). This means that tissue with a high oxygen consumption will show steep oxygen gradients. It is obvious that a large number of additional microcirculatory factors (e.g., intercapillary distance, capillary radius, capillary flow, capillary oxygen content, red blood cell size, adherence of cellular blood components, oxygen diffusion coefficient, etc.) regulate the available amount of molecular oxygen for the cell. One decisive factor is the diffusion distance, which is inversely related to capillary density. Diffusion distance is increased by thickening of the muscle fibers (hypertrophy) or closure of some capillaries. On the other hand, tissue oxygenation may be improved by shortening the diffusion distance caused by the opening of closed capillaries (a fast process occurring in exercising muscle) or by formation of new capillaries (a slow process taking weeks and possibly occurring in high altitude adaptation). The microcirculation is subjected to neural, hormonal, metabolic and physical regulatory factors, as illustrated by capillary flow heterogeneity in time and in space observed in *in vivo* studies (Gonzalez and Bassingthwaighte [8]). Consequently, tissue oxygenation, cannot be adequately expressed by a mean tissue pO_2 value because there exists in fact a pO_2 field with widely varying local pO_2 values. That is to say, a normal mean pO_2 value may suggest a spurious normal oxygenation, while not revealing local regions of hypoxia or anoxia. For this reason it is mandatory to have knowledge about a large number of pO_2values (e.g., 100 or 200) from the intercellular space in order to construct a tissue pO_2 profile. A tissue pO_2 profile is expressed by a pO_2 frequency distribution curve, a pO_2 histogram, for an overall representation of molecular oxygen in the tissue, available for the cellular metabolism. The use of a cumulative pO_2 histogram allows, e.g., calculation of a median value (P50), a 10% percentile, etc. Several deviations from a normoxic pO_2 histogram can be recognised: a left shift to hypoxia, a right shift to hyperoxia, hypoxic or normoxic homogeneous c.q. heterogeneous distribution. In the next paragraph several methods to assess tissue oxygenation are presented with the emphasis on the clinical application before, during and after hyperbaric oxygen therapy.

3. Methodology

Some methods to measure tissue oxygenation are meant for *in vitro* studies, whereas others can be used in clinical practice. In general, polarographic and non-polarograhic methods can be distinguished (Table 1).

4. Non-polarographic techniques

4.1. Laser-Doppler flowmetry

Laser-Doppler flowmetry (LDF), an indirect method to assess tissue oxygenation, measures dermal bloodflow over a 1 mm^3 area of measurement with an approximate depth of 1.5 mm. Light from a helium–neon laser scattered from tissue *in vivo* is broaded in line width as a result of the Doppler shift produced by moving cells in the microcirculation. Clinically severe skin ischemia may exist with an adequate laser-Doppler flow signal caused by uneven capillary flow distribution, mainly occurring in A-V anastomosis (Kvernebo and Seem [25]). Hammerlund et al. [9] used the LDF to evaluate the effects of oxygen breathing under hyperbaric conditions during an 8-min exposure time. He reported a flow decrease induced by hyperbaric oxygenation. However, most of the LDF signal (>80%) is dominated by non-nutritional, thermo-regulatory, shunt vessels of the skin (Bollinger and Fagrell [1]). The findings of this study are therefore mainly indicative of a decreased flow in non-nutritional shunt vessels and not a decreased flow in nutritional capillaries. Intravital capillary microscopy of the nailfold provides information about nutritional capillary blood flow, in patients with compromised skin oxygenation (Jacobs et al. [18]). We studied the influence of HBO on nutritional capillary blood flow in healthy persons by intravital nailfold microscopy (van der Kleij et al. [20,21]) and observed after a longer exposure time (20 min) to HBO the onset of a significant increase of red blood cell velocity (Vrbc) in nutritional capillaries accompanied by significantly increased transcutaneous partial oxygen tension and a significantly decreased temperature of the hand. These results are indicative of the necessity to observe longer periods during exposition to HBO. Furthermore, the LDF method only can lead to inappropriate conclusions.

Table 1

Polarographic and non-polarographic methods to assess tissue oxygenation. (For an overview, see Stone et al. [31] and Hofer [10])

Polarographic techniques	Non-polarographic techniques
Tissue tonometry	
1. One-way/two-way tonometer	1. Laser-Doppler flowmetry
2. With/without electrode inserted	2. Mass spectroscopy
Transcutaneous electrode	3. Phosphorescence quenching (Wilson)
Needle electrodes	4. NADH fluorescence (Ince et al. [17])
1. Mono/bipolar electrodes	5. Near-infrared spectroscopy (Elwell et al.)
2. Micro/macro-electrodes	6. Magnetic resonance spectroscopy (Stone et al. [32])
3. Open or bare type	7. Electron spin resonance (Stone et al. [32])
4. “Recessed” type	8. Positron emission tomography (Stone et al. [32])
5. Flow type	9. Frozen specimen spectroscopy (Gayeski and Honig [7])

4.2. Mass spectroscopy (MSP)

Brantigan et al. [2] reported the development of a heparinized silicone rubber diffusion membrane and catheter for continuous *in vivo* measurement of blood gas tensions by mass spectrometry. The 99% response time for oxygen was 5.1 min and for carbon dioxide 9.9 min. The membrane end of the catheter was inserted into the tissue and the other end was connected to the vacuum system of a mass spectrometer, and 5×10 ml/s of gas was continuously withdrawn. This yielded mean intramyocardial O_2 and CO_2 tensions in dogs of 18 ± 5 mm Hg and 40 ± 14 mm Hg, respectively. Detection of inadequate tissue oxygenation with this method is possible. The disadvantage of MSP is the slow response time in comparison with other methods. MSP has been successfully used in experimental and clinical studies. The clinical usefulness of MSP as a routine method remains controversial and is restricted to more specialised centres.

4.3. Optical methods and others

The meeting report of a workshop entitled "Oxygen in Human Tumours: Correlation between Methods of Measurements and Response to Therapy" (Stone et al. [32]) gave a good overview of other methods to assess tissue oxygenation such as: phosphorescence quenching, NADH fluorescence, near-infrared spectroscopy, magnetic resonance spectroscopy, electron spin resonance, positron emission tomography, and frozen specimen spectroscopy. Issues such invasiveness, space resolution, what does it measure?, start-up costs and estimated cost/study, etc. were presented. It is beyond the scope of this short communication to go into further detail. In general, these promising methods are in an early stage of development, prohibitively expensive or complicated and consequently not yet suitable for routine clinical application.

5. Polarographic techniques

Polarography was mainly developed by Heyrovsky and Shikata [10] who used a "dropping mercury" electrode to measure dissolved ions and realized that a dropping mercury electrode could provide information about dissolved ions. Dissolved oxygen can be reduced by electrolysis in the presence of a noble metal (Pt, Au, Ag). Polarographic oxygen determination involves the use of electrolysis for measuring electro-reducible oxygen. An electric current is generated, characterized by a current voltage curve with a plateau proportional to the concentration of dissolved oxygen. Even in 1942 the need to measure local oxygen pressure *in vivo* led to the development of solid Pt microelectrodes by Davies and Brink. They described a "recessed-type" electrode to simplify diffusion geometry and to enable measurement of the absolute oxygen tension in tissues (Davies [4]). The "open-tip" microelectrode, in which a Pt wire is in direct contact with the analysed medium, may be used to monitor the oxygen concentration changes in biological media but is not suitable for exact analysis of absolute oxygen tension (Kreuzer et al. [24]). The cathode of the above-mentioned electrode is a Pt wire and the anode is usually an Ag/AgCl reference electrode. In 1956 Clark introduced a system which enclosed cathode, anode, and electrolyte behind a hydrophobic membrane separating the entire measuring system from the medium. The polarographic principle has been used for the determination of oxygen in blood, for continuously recording oxygen pressure *in vivo* and for oxygen measurements in tissues.

5.1. Two/one-way tissue tonometry with/without insertion of a polarographic oxygen sensor

Another technique to measure tissue pO_2 involves the placement of a compartment in the tissue filled with anoxic fluid and freely permeable to gases. After a certain interval the pO_2 in the compartment fluid

is in equilibrium with the pO_2 of the surrounding tissue and can subsequently be determined *ex vivo* or *in vivo*. This method is called tonometry. Hunt [16] modernized this method by introducing a "teflon" tube in the tissue via a stainless steel hypodermic needle. Niinikoski and Halkola [28] advocated the implantation of silicone rubber tubes into the muscle tissue for continuous monitoring of muscle pO_2 and pCO_2. On one side the tube is perfused with hypoxic saline (rate 0.07 ml/min) and the other side it is connected to a pO_2 electrode as used for blood gas analysis. High permeability of silicone rubber for oxygen allows fast equilibration between skeletal muscle pO_2 and pCO_2 and tube content. The same method was used by Jussila et al. [19] to evaluate re-vascularization surgery. A 16-cm long silicone rubber tube was inserted into the calf musculature and pre-, per-, and postoperative skeletal muscle gas tensions were obtained. After implantation of the silicone rubber tube, a hyperemic tissue reaction was observed, which was constant, reproducible, and disappeared after three days. Littooy et al. [26], using the same method, determined subcutaneous tissue oxygen pressure and came to the same conclusions as Niinikoski and Halkola [28] that tissue tonometry can be used to assess tissue perfusion but one must realize that no information about the pO_2 distribution in the tissue is obtained. The next step was the insertion of a 25-m polarographic pO_2 electrode inside the silastic tubing. A one-way tissue tonometer was born and provided stable data for 48 h (Rabkin et al. [29]). A commercially available (Continucath) one-way polarograhic tonometer was tested by Hofer and can be used to monitor free flap viability (Hofer et al. [12]). Inserted into skeletal muscle tissue consistent and accurate absolute mean pO_2 values can be obtained.

5.2. Transcutaneous pO_2 electrodes

Transcutaneous polarographic pO_2 electrodes ($PtcO_2$) were developed for noninvasive monitoring of arterial pO_2. The clinical value has been proven in newborn infants (Huch and Huch [14]). The modified Clark electrode (Huch et al. [15]) contains a heating element and a thermistor. The sensor is attached to the skin by an adhesive fixation ring filled with a contact solution. Heating the skin to 44–45°C produces vasodilatation of the underlying arterioles and capillary bed under the sensor, increases the size of the skin pores, and shifts the oxyhemoglobin curve to the left. These events induce increased blood flow, diffusion of oxygen through the skin and the release of oxygen from haemoglobin, respectively. The oxygen tension measured by the sensor closely approximates the capillary pO_2 value. In older patients $PtcO_2$ values vary with age and clinical conditions caused by physiological changes of skin lipid structures and the oxyhaemoglobin curve (Shoemaker and Vidyasagar [30]). The transcutaneous pO_2 values are influenced by local flow conditions, arterial pO_2 and skin conditions. In particular, the heat applied in this technique interferes with the normal vasoconstrictive reflexes and alters the physiology of the heated region during the measuring process. A large scatter of "normal" pO_2 values (Franzeck et al. [6]) makes interpretation rather difficult. The clinical threshold to use this method is low and therefore the method is widely used to assess the severity of peripheral vascular occlusive disease, to select the level of amputation in cases of critical limb ischemia, to monitor tissue viability of skin flaps (Wyss et al. [34]), or vascular reconstructive procedures (Urk and Feenstra [33]) and to predict the outcome of HBO therapy for problem wounds (Mathieu et al. [27]).

5.3. Micro and macro polarographic pO_2 needle electrodes

From a clinical point of view a needle pO_2 electrode must fulfill several conditions: (1) "easy" handling, (2) reliable, (3) instantaneous measurement results.

A micro pO_2 electrode might be ideal for tissue oxygen measurement but unfortunately it is too fragile for routine clinical application. The incorporation of a microelectrode in a silver needle or a stainless steel needle provides a pO_2 electrode with an outside diameter corresponding to the outside diameter of the needle. This type of needle electrode is rugged enough to be used in clinical situations. Using a needle pO_2 electrode a number of pO_2 values can be obtained by stepwise penetration into the tissue (Fleckenstein and Weiss [5]) or stepwise withdrawal out of the tissue (van der Kley et al. [20,21]). Fleckenstein and Weiss [5] showed that a pO_2 needle electrode enables the construction of a pO_2 histogram of skeletal muscle which is comparable with a pO_2 histogram obtained with a multi-wire surface electrode. Most polarographic pO_2 needle electrodes are custom made. Since a few years a polarographic computerised pO_2 measurement unit is commercially available (pO_2-Histograph, Eppendorf, Hamburg, Germany). The probe is automatically advanced through the tissue in a so-called "pilgrim step process". We used this pO_2 unit to assess the skeletal muscle tissue pO_2 during hyperbaric oxygen therapy in healthy volunteers and in patients. After calibration during normobaric conditions the pO_2-Histograph was pressurised to 3 ATA and the calibration procedure was repeated. The increased ambient pressure did not affect the function of the pO_2-Histograph.

The use of a commercially available computerised pO_2 unit allows comparison of the results with different investigators and with values derived from the literature.

Furthermore, a better patient stratification can be achieved from the results found by different hyperbaric units.

6. General conclusion

It may be concluded that polarographic transcutaneous pO_2 measurements and polarographic skeletal muscle pO_2 measurements provide reliable information for assessment of tissue oxygenation. It allows (pre)stratification of patients who will benefit from a new therapeutic regimen. Promising, less invasive methods to assess tissue oxygenation are in a developmental stage and may be more suitable for routine clinical application in the near future and less expensive.

References

[1] A. Bollinger and B. Fagrell, Dynamic capillaroscopy without dyes, in: *Clinical Capillaroscopy*, A. Bollinger and B. Fagrell, eds, Hogrefe and Huber, Toronto, 1990, pp. 9–30.
[2] J.W. Brantigan, V.L. Gott and M.N. Martz, A teflon membrane for measurement of blood and intramyocardial gas tensions by mass spectroscopy, *J. Appl. Physiol.* **32**(2) (1972), 276–282.
[3] P.W. Davies and F. Brink, Jr., Microelectrodes for measuring local oxygen tension in animal tissues, *Rev. Sci. Instrum.* **13** (1942) 524–533.
[4] P.W. Davies, The oxygen cathode, in: *Physical Techniques in Biological Research*, Vol. IV, W.L. Nastuk, ed., Academic Press, New York, 1962, pp. 137–179.
[5] W. Fleckenstein and Ch. Weiss, A comparison of pO_2-histograms from rabbit hind-limb muscles obtained by simultaneous measurements with hypodermic needle electrodes and with surface electrodes, *Adv. Exp. Med. Biol.* **169** (1984), 445–447.
[6] U.K. Franzeck, P. Talke, E.F. Bernstein, F.L. Golbranson and A. Fronek, Transcutaneous pO_2 measurements in health and peripheral arterial occlusive disease, *Surgery* **91** (1982), 156–163.
[7] T.E.J. Gayeski and C.R. Honig, O_2 gradients from sarcolemma to cell interior in a red muscle at maximal VO_2, *Am. J. Physiol.* **251** (1986), H789–H799.
[8] F. Gonzalez and J.B. Bassingthwaighte, Heterogeneities in regional volumes of distribution and flows in rabbit heart, *Am. J. Physiol.* **258** (1990), H1012–H1024.
[9] C. Hammerlund, J. Castenfors and P. Svedman, Dermal vascular response to hyperoxia in healthy volunteers, in: *Hyperbaric Medicine Proceedings on the 2nd Swiss Symposium on Hyperbaric Medicine*, D.J. Bakker and J. Schmutz, eds, 1988, pp. 55–59.

[10] J. Heyrovsky and M. Shikata, Researches with the dropping mercury cathode, *Rec. Trav. Chim. Pays Bas* **44** (1925), 496–499.
[11] S.O.P. Hofer, Tissue oxygen tension an indicator of tissue perfusion, Thesis, University of Amsterdam, 1993.
[12] S.O.P. Hofer, E.J.F. Timmenga, R. Christiano and K.E. Bos, An intravascular oxygen tension monitoring device used in myocutaneous transplants: a preliminary report, *Microsurgery* **14** (1993), 304–309.
[13] S.O.P. Hofer, A.J. van der Kleij and K.E. Bos, Tissue oxygenation measurement: a directly applied Clark-electrode in muscle tissue, *Adv. Exp. Med. Biol.* **317** (1992), 779–784.
[14] A. Huch and R. Huch, Klinische und physiologische Aspekte der transcutanen Sauerstoffdruckmessung in der Perinatalmedizin, *Z. Geburtsh. Perinat.* **179** (1979), 235–249.
[15] R. Huch, D.W. Lübbers and A. Huch, Quantitative continuous measurements of partial oxygen pressure on the skin of adults and new born babies, *Pflügers Arch.* **337** (1972), 185–198.
[16] T.K. Hunt, A new method of determining tissue oxygen tension, *Lancet* **2** (1964), 137–1371.
[17] C. Ince, J.M.C.C. Coremans and H.A. Bruining, *In vivo* NADH fluorescence, *Adv. Exp. Med. Biol.* **317** (1992), 277–296.
[18] M.J.H.M. Jacobs, D.Th. Ubbink, P.J.E.H.M. Kitslaar, J.H.M. Tordoir, D.W. Slaaf and R.S. Reneman, Assessment of the microcirculation provides additional information in critical limb ischemia, *Eur. J. Vasc. Surg.* **6** (1992), 135–141.
[19] E.J. Jussila, J. Niinikoski and E. Vattinen, Intraoperative recording of tissue gas tensions in calf muscles of patients with peripheral arterial disease, *J. Surg. Res.* **29** (1980), 535–540.
[20] A.J. van der Kleij, H. Vink, Ch.P. Henny, D.J. Bakker and J.A.E. Spaan, Red blood cell velocity in nailfold capillaries during hyperbaric oxygenation, Presented at 20th Annual Meeting International Society on Oxygen Transport to Tissue, Mainz, Germany, *Adv. Exp. Med. Biol.* (1994, in press).
[21] A.J. van der Kleij, J. de Koning, G. Beerthuizen, R.J.A. Goris, F. Kreuzer and H.P. Kimmich, Early detection of hemorrhagic hypovolemia by muscle oygen pressure assessment: Preliminary report, *Surgery* **93**(4) (1983), 518–524.
[22] C.G.H.M. Kooiman, A.J. van der Kleij, Ch.P. Henny, M.S. Dongelmans and M.S. Günderoth, Effects of isovolemic hemodilution on microcirculatory parameters and tissue oxygenation during anaesthesia, Presented at the 21th Annual meeting of the International Society on Oxygen Transport to Tissue, August 14–18, 1993, San Diego, USA, *Adv. Exp. Med. Biol.* (in press).
[23] K.H. Kopp, M. Kieser and E. Sinagowitz, Muscle pO_2 measurement in critically ill patients and its correlation to cardiac output and arterial pO_2, in: *Monitoring of Vital Parameters During Extracorporeal Circulation*, H.P. Kimmich, ed., Karger, Basel, 1981, pp. 87–94.
[24] F. Kreuzer, H.P. Kimmich and M. Brezina, Polarographic determination of oxygen in biological materials, in: *Medical and Biological Applications of Electrochemical Devices*, Vol. 6, J. Koryta, ed., 1980, pp. 173–261.
[25] K. Kvernebo and E. Seem, Erythromelalgie – pathophysiological and therapeutic aspects; a preliminary report, *J. Oslo City Hosp.* **37** (1987), 9–12.
[26] F. Littooy, R. Fuchs, T.K. Hunt and G.F. Sheldon, Tissue oxygen as a real-time measure of oxygen transport, *J. Surg. Res.* **20** (1976), 321–325.
[27] D. Mathieu, F. Wattel, G. Bouachour, V. Billard and J.F. Defoin, Post-traumatic limb ischemia: Prediction of final outcome by transcutaneous oxygen measurement in hyperbaric oxygen, *J. Trauma.* **30** (1990), 307–314.
[28] J. Niinikoski and L. Halkola, Skelatal muscle pO_2: indicator of peripheral tissue perfusion in haemorrhagic shock, in: *Transport to Tissue III*, I.A. Silver, M. Erecinska and H.I. Bicher, eds, Adv. Exp. Med. Biol., Vol. 94, Plenum Press, New York, 1978, pp. 585–592.
[29] J. Rabkin, R. Alena, J. Morse, R. Goodson III and T.K. Hunt, Oxygen tension measurements using an oxygen polarographic electrode sealed in an implantable silastic tonometer: a new technique, *Adv. Exp. Med. Biol.* **222** (1988), 267–273.
[30] W.C. Shoemaker and D. Vidyasagar, Physiological and clinical significance of $PtcO_2$ and $PtcCO_2$ measurements (editorial), *Crit. Care Med.* **9** (1981), 689–690.
[31] I.A. Silver, Heterogeneity in tissue oxygenation; systemic and local factors, in: *Oxygen Transport to Tissue*, A.G.B. Kovach, E. Dora, M. Kessler and I.A. Silver, eds, Adv. Physiol. Sci., Vol. 25, Pergamon Press, Oxford, 1981, pp. 67–76.
[32] H.B. Stone, J.M. Brown, T.L. Phillips and R.M. Sutherland, Oxygen in human tumors: correlations between methods of measurement and response to therapy, Meeting report, *Rad. Res.* **136** (1993), 422–434.
[33] H. van Urk and W.A. Feenstra, What can transcutaneous oxygen measurements tell us?, in: *Limb Salvage and Amputation for Vascular Disease*, R.M. Greenhalg, C.W. Jamieson and A.N. Nicolaides, eds, W.B. Saunders, Philadelphia, 1988.
[34] C.R. Wyss, F.A. Matsen III, C.W. Simmons and E.M. Burgess, Transcutaneous oxygen tension measurements on limbs of diabetic and nondiabetic patients with peripheral vascular disease, *Surgery* **95** (1984), 339–345.

Hemorheology and tissue oxygen transport during hypertension

G. Cicco and A. Pirrelli
DIMO, Internal Medicine and Hypertension, University of Bari, Bari, Italy

1. Introduction

Studies in hemorheology and microcirculation have seen many important developments in the last few years, strengthening the link between scientific research and clinical practice (for example, in the study and treatment of peripheral arterial occlusive disease (POAD), neoplasm, shock, hypertension, diabetes, smoking, lipoproteinosis, uraemia, sickle cell hemoglobin C disease, polycytemia, malaria, etc.). In these pathologies there are various microcirculatory alterations. Among these the following factors are important: capillary proliferation, capillary rarefaction, vascular tortuosity, capillary plugging, endothelial inflammation, microaneurysm, hyperviscosity, red blood cell deformability, erythrocyte and platelet aggregability, leukocyte rigidity, vascular elasticity and the condition of the arterial wall and capillary basal membrane [1].

2. Vascular and hemorheological alterations during hypertension

During hypertension various vascular and hemorheological alterations are possible. These alterations are caused by is reduced compliance in the arterial and venous system with consequent change in the large arteries and veins, increase in vascular tone, increase in vessel wall thickness, contemporary hypertrophy of the vascular smooth muscle cells (VSMC) and alterations in the composition of the wall extracellular matrix [2–4]. At the microcirculatory level in the smaller arteries there is an increase in the blood flow resistance. The main functions of the microcirculation are tissue perfusion, tissue oxygenation and at the same time nutrient intake and clearing of the tissue catabolites. New arterioles can be formed from the endothelial cells which coat the capillaries to meet increased tissular requirements or to substitute small regressed vessels [5,6]. In borderline hypertension the diameter of the conjunctival arterioles is increased with respect to normal blood pressure levels, whilst in essential hypertension of a long duration this does not occur and there is, instead, vasoconstriction without hypertrophy of vascular smooth muscle cells [7]. In secondary hypertension (for example, in experimental forms of renal hypertension or salt-dependent hypertension) there is a reduction in vessel diameter plus a reduced number of capillaries (rarefaction), but in a less generalised form compared with that found in essential hypertension [8].

Microvascular rarefaction, i.e., the reduced proliferation of endothelial cells, is a pathogenic mechanism found in essential hypertension [9]. It is possible to have a first stage alteration of the functional type with vasoconstriction, followed by a stage of structural alteration with an obliteration of the microcirculatory capillaries [10]. In this field hemorheological alterations definitely play an important role. For example, an increase in blood and plasma viscosity, an increased erythrocyte aggregation and a reduction in erythrocyte deformability are often present in hypertensives, especially if they are not being treated. Up until now these alterations have not been systematically studied as possible pathogenic factors in hypertension [2]. In fact, there have been many more studies dealing with the vessels than with blood [11–15].

During hypertension great importance is also given to a reduced peripheral oxygenation which itself indirectly indicates a reduction in peripheral perfusion. Therefore, in this field it is important to study

oxygen transport, release in the tissues, as well as hemoglobin function. On the other hand, hemoglobin is involved in vascular tone modulation indirectly. In fact, hemoglobin possesses a high affinity (at least 10,000 times higher than that for oxygen) for nitric oxide (NO), a strong vasodilative substance. At a peripheral level, the red blood cells release O_2 and the hemin group releases NO to endothelial receptors via thiol groups. In this way NO can be used to modulate the blood pressure by inducing vasodilatation in microcirculation [16,17].

3. Tissue oxygenation and oxygen assessment

The factors which influence oxygen transport and delivery depend on lung function, cardiac output and hemoglobin content. Other factors are vascular resistance, blood viscosity and perfusion pressure. In microcirculation the number of functioning capillaries, capillary surface area and capillary hematocrit can influence tissue oxygenation. Other important factors at an intracellular level are the amount of ATP produced by mitochondria and the mitochondrial oxidative phosporylation level [18,19].

A direct quantitative assessment of tissue oxygen supply and tissue perfusion is provided by continuous PO_2 measurement, which can be performed in many ways in clinical and research conditions.

4. Transcutaneous oxymetry: our experience

We used transcutaneous oxymetry to evaluate peripheral oxygen availability and peripheral perfusion in hypertensives. In hypertensive subjects, especially non-treated, we obtained TCP O_2 values lower than those found in normal control subjects. We first used a single transcutaneous oxymeter (Microgas 7640 MK2, Kontron Instruments) with a Combi sensor to measure PO_2 and PCO_2 levels contemporarily in the subclavian area. We studied 18 hypertensive smokers (10 male and 8 female, aged 55 ± 6 years) with left ventricular hypertrophy, hyperfibrinogenemia and peripheral occlusive arterial disease (POAD II a). After a 120-day oral treatment with an Amlodipine (10 mg OD), antihypertensive drug (Ca antagonist) and Defibrotide 400 mg OD (a hemorheological drug), we noted normalisation of blood pressure (BP) values, improvement in $TCPO_2$ and Regional Perfusion Index (RPI), increase in the ankle/brachial pressure index and normalisation in fibrinogenemia [20,21].

We also carried out studies using 3 oxymeters together in the subclavian area and the calves. One study in 21 hypertensive smokers (11 male and 10 female, aged 50 ± 5 years with POAD IIa) evaluated $TCPO_2$, RPI (Calf $TCPO_2$/Subclavian $TCPO_2$) and Trans Cutaneous Index (TCI = TCP O_2/Pa O_2) [22]. Following a 90-day treatment with the previously mentioned medication we noted an improvement in BP values and peripheral oxygen delivery [23]. A further study was realised under baseline conditions and during physical exercise (step test) on 22 non-smokers (12 male and 10 female, aged 55 ± 4 years, lipoproteinosis and POAD IIa). After 90 days we saw an improvement in BP values and $TCPO_2$ before and after the step test [24].

5. Conclusion

After antihypertensive treatment we noted an improvement in $TCPO_2$ and peripheral perfusion. We can consider the transcutaneous oxymetry as a good, non-invasive, easily repeatable technique to evaluate oxygen delivery and peripheral perfusion, especially in patients with hypertension complicated by smoking, diabetes, lipoproteinosis and/or arterial occlusive disease (POAD) I or IIa.

References

[1] M. Intaglietta, in: *Microcircolazione e Microangiologia*, F. Pratesi, ed., Minerva Medica, Vol. VII, 1990.
[2] H.A.J. Struijker Boudier, Sviluppo vascolare ed ipertensione, in: *Textbook of Hypertension*, Ital. Momento Medico, Vol. 10, 1995, pp. 191–203.
[3] B.I. Levy, J. Benessiano, P. Poitevin and M.E. Safar, Endothelium-dependent mechanical properties of the carotid artery in WKY and SHR, role of angiotensin converting enzyme inhibition, *Circ. Res*. **66** (1990), 321–328.
[4] R. Lee, Structural and functional consequence of antihypertensive treatments on blood vessels, in: *Blood Vessel Changes in Hypertension: Structure and Function*, R. Lee, ed., CRC Press, Boca Raton, FL, 1989, pp. 169–190.
[5] O. Hudlicka, What makes blood vessels grow?, *J. Physiol*. **444** (1991), 1–24.
[6] R.N. Feinberg, G.K. Sherer and R. Auerbach, eds, *The Development of the Vascular System*, Karger, Basel, 1991.
[7] J.M. Sullivan, R.L. Prewitt and J.A. Josephs, Attenuation of the microcirculation in young patients with high output borderline hypertension, *Hypertension* **5** (1983), 844–851.
[8] G.A. Meininger, P.D. Harris and I.G. Joshua, Distribution of microvascular pressure in skeletal muscle of one-kidney, one-clip, two-kidney, one-clip, and deoxycorticosterone-salt hypertensive rats, *Hypertension* **6** (1984), 26–34.
[9] P.M. Hutchins and A.E. Darnell, Observation of a decreased number of small arterioles in spontaneously hypertensive rats, *Circ. Res*. **34/35**(1) (1974), 161–165.
[10] R.L. Prewitt, H. Hashimoto and D.L. Stack, Structural and functional rarefaction of microvessels in hypertension, in: *Blood Vessel Changes in Hypertension: Structure and Function*, R. Lee, ed., CRC Press, Boca Raton, FL, 1990, pp. 71–90.
[11] G. Cicco and A. Pirrelli, Emoreologia clinica ed ipertensione arteriosa, *Minerva Cardioangiol*. **41** (1993), 213–223.
[12] M.P. Lorient-Roudaut, R. Roudaut and G.L. Freyburger, Hemorheological abnormalities in essential hypertension, *Clin. Hemorheol*. **7** (1987), 537.
[13] S. Chien, Blood rheology in hypertension and cardiovascular disease, *Cardiovasc. Med.* **2** (1977), 356–360.
[14] A. Petralito, L.S. Malatino and C.E. Fione, Erythrocyte aggregation in different stages of arterial hypertension, *Thromb. Hemost*. **54** (1985), 555.
[15] G.C. Maggi, R. Zacco and M. Ornaghi, La filtrazione eritrocitaria nella ipertensione arteriosa, *Ric. Clin. Lab*. **15**(1) (1985), 43.
[16] J.L.A. Li, C. Bonaventura, J. Bonaventura and J.S. Stamler, S-nitrosohemoglobin: a dynamic activity of blood involved in vascular control, *Nature* **380** (1996), 221–226.
[17] J. Glanz, Hemoglobin reveals new role as blood pressure regulator, *Science* **271** (1996), 1670.
[18] C. Ince, Determinants of tissue oxygenation, in: *Tumor Oxygenation and Radiotherapy*, Symposium AMC, University of Amsterdam, 1996.
[19] A.J.van der Kleij and A.D. Bakker, Oxymetry, in: *Handbook of Hyperbaric Medicine*, G. Oriani, A. Marroni and F. Wattel, eds, Springer, Milano, 1996, pp. 670–685.
[20] G. Cicco, E. Dolce, G. Gigante and A. Pirrelli, Ossimetria transcutanea in fumatori ipertensi moderati con vasculopatia periferica in trattamento con amlodipina e defibrotide e abolizione del fumo di sigaretta, *Minerva Cardioangiol*. **43** (1995), 423–428.
[21] G. Cicco, C. Di Perri, G. Gigante, P. Vicenti and A. Pirrelli, The transcutaneous oxygen evaluation after 120 days of amlodipine and defibrotide treatment in hypertensive smokers, in: *3rd Int. Workshop IASACO*, Monte Carlo, 1995, p. 92.
[22] M.W. Webster and D.L. Steed, $TCPO_2$ theory and clinical applications, in: *Vascular Medicine*, H. Boccalon, ed., Elsevier, Amsterdam, 1993, p. 501.
[23] G. Cicco, A.J. van der Kleij, P. Vicenti and A. Pirrelli, Transcutaneous oxymetry in hypertensive smokers treated for 90 days with amlodipine and defibrotide, *Microcirc. Off. J. Chinese Soc. Microcirc*. (1995), 105–159.
[24] G. Cicco, P. Vicenti, A. Pirrelli and A.J. van der Kleij, Peripheral perfusion and tissue oxygenation improvement induced by antihypertensive medication combined with lipoproteinosis, in: *XVIII ISOTT Congress*, Pittsburgh, PA, 1995, p. 63.

New possibilities in transcutaneous oxymetry technology

P.M. Lugarà
Dipartimento di Fisica – Unità I.N.F.M., via E. Orabona 4, I-70126 Bari, Italy

Abstract. The applicability of non-invasive *in vivo* spectroscopic techniques to measure the muscle oxygenation through the skin, by determining the relative concentration of oxygenated hemoglobin, is discussed. A simple set-up, based on commercially available near-infrared light emitting or laser diodes, is presented. This could result in the development of user-friendly systems for wide clinical use.

1. Introduction

In the near-infrared region, from 700 to 900 nm, light penetrates several centimetres into living tissues. In principle, spectroscopic methods could then be used to detect the presence and to measure the concentration of tissue constituents, such as hemoglobin, which absorb in that spectral region. In order to obtain quantitative information about important biological parameters, namely the concentrations of oxygenated and deoxygenated hemoglobin, the problem arising from the highly scattering behaviour of the living tissues must be overcome.

2. Tissue optical properties

In strongly scattering media the light propagation can be described by solving the properly written [1] diffusion equation for photons:

$$\left[\delta\Phi(r,t)/\delta t\right]/c_{\mathrm{n}} - D\nabla^2\Phi(r,t) + \mu_{\mathrm{a}}\Phi(r,t) = S(r,t), \tag{1}$$

where c_{n} is the light speed in the medium, Φ is the photon fluence rate at position r and time t, μ_{a} is the absorption coefficient, S is the source term and the diffusion coefficient D is given by $D = \{3[\mu_{\mathrm{a}} + (1-g)\mu_{\mathrm{s}}]\}^{-1}$, μ_{s} being the diffusion coefficient and g the mean cosine of the scattering angle. General expressions for the transmittance $T(\rho,d,t)$ and the reflectance $R(\rho,t)$ of a slab of thickness d, in points at a distance ρ from the source on the sample surface, have been obtained [2] for light pulses shorter than 0.1 ns. In this case, from the experimental point of view, the time-resolved diffused reflectance carries information on both μ_{a} and μ_{s}, since: (i) the time derivative $\delta \ln R/\delta t$ asymptotically approaches the quantity $\mu_{\mathrm{a}}c_{\mathrm{n}}$ at long delay times, i.e., at least 100 times the pulse duration and then more than 10 ns after the light pulse; (ii) at the time $t_{\max}$, corresponding to the maximum detected signal,

$$(1-g)\mu_{\mathrm{s}} = \left(4\mu_{\mathrm{a}}c_{\mathrm{n}}^2t_{\max}^2 + 10c_{\mathrm{n}}t_{\max}\right)/3\rho^2 - \mu_{\mathrm{a}}. \tag{2}$$

In a different approach, involving the use of continuous wave light sources, sinusoidally modulated at a frequency f, the information on absorption and scattering coefficients is carried [1] by the phase shift q between exciting and reflected light, according to the simplified equation

$$\theta = a\rho\left[(1-g)\mu_{\mathrm{s}}f\right]^{1/2}\left(1 - \mu_{\mathrm{a}}c_{\mathrm{n}}/(4\pi f)\right), \tag{3}$$

where a is a constant given by $(6\pi/c_{\mathrm{n}})^{1/2}\sin(\pi/4)$. Better results are achieved, in terms of signal-to-noise ratio, if the double modulation technique is adopted, with f of the order of 100 MHz and the light detector gain modulated at a frequency $f + \Delta f$, with Δf below 1 MHz.

Nevertheless, only time-resolved spectroscopy seems to be able to give directly the absorption coefficient of the investigated species; but its set-up is expensive, cumbersome and could be difficult to use. Indeed, if the dual-wavelength spectroscopic methods are adopted, the problem with become much simpler, as we will show for oxygenated hemoglobin.

3. Non-invasive optical oxymetry

At two near-infrared wavelengths λ_1 and λ_2, for which the absorbance of water and cytochrome is negligible if compared with that of hemoglobin, the absorption coefficients of the latter can be written as:

$$\mu_{a,1} = \varepsilon_{1,\mathrm{Hb}}[\mathrm{Hb}] + \varepsilon_{1,\mathrm{HbO_2}}[\mathrm{HbO_2}],$$

$$\mu_{a,2} = \varepsilon_{2,\mathrm{Hb}}[\mathrm{Hb}] + \varepsilon_{2,\mathrm{HbO_2}}[\mathrm{HbO_2}],$$

where ε_1 and ε_2 are the extinction coefficients and [Hb] and [$\mathrm{HbO_2}$] are the tissue concentrations of oxy- and deoxygenated forms of hemoglobin. If the hemoglobin saturation is given by $Y = [\mathrm{HbO_2}]/\{[\mathrm{Hb}] + [\mathrm{HbO_2}]\}$, then

$$\mu_{\mathrm{a},1}/\mu_{\mathrm{a},2} = \{\varepsilon_{1,\mathrm{Hb}} + Y[\varepsilon_{1,\mathrm{HbO_2}} - \varepsilon_{1,\mathrm{Hb}}]\}/\{\varepsilon_{2,\mathrm{Hb}} + Y[\varepsilon_{2,\mathrm{HbO_2}} - \varepsilon_{2,\mathrm{Hb}}]\}. \tag{4}$$

Furthermore, if λ_2 is the isobestic wavelength of hemoglobin, just above 800 nm, the last equation becomes

$$\mu_{\mathrm{a},1}/\mu_{\mathrm{a},2} = \varepsilon_{1,\mathrm{Hb}}/\varepsilon_{2,\mathrm{Hb}} + Y[\varepsilon_{1,\mathrm{HbO_2}} - \varepsilon_{1,\mathrm{Hb}}]/\varepsilon_{2,\mathrm{Hb}}. \tag{5}$$

This shows that the ratio of the absorption coefficients of hemoglobin at two near-infrared wavelengths is a linear function of the tissue hemoglobin saturation Y and is independent of the total hemoglobin concentration. If time-resolved spectroscopy measurements on tissue hemoglobin are carried out with the dual-wavelength technique, for time delays much longer than t_{max}, the ratio of absorption coefficients is:

$$\mu_{\mathrm{a},1}/\mu_{\mathrm{a},2} = (\delta \ln R_1/\delta t)/(\delta \ln R_2/\delta t). \tag{6}$$

By taking into account the possibility to select the two near-infrared wavelengths so that the corresponding scattering coefficients of the tissue differ negligibly, the application of the dual-wavelength method to the modulation spectroscopy results in a simple dependence of the phase–shift ratio on the absorption coefficients:

$$\theta_1/\theta_2 = \big(1 - \mu_{\mathrm{a},1}c_{\mathrm{n}}/(4\pi f)\big)/\big(1 - \mu_{\mathrm{a},2}c_{\mathrm{n}}/(4\pi f)\big). \tag{7}$$

If different phase shifts due to scattering and background absorption must be taken into account, the equation can be rewritten as directly relating the absorption coefficient ratio to the phase shift one:

$$(\theta_1 - \theta_{1,s})/(\theta_2 - \theta_{2,s}) = \mu_{a,1}/\mu_{a,2}. \tag{8}$$

However, it is much more interesting to consider that, in the near-infrared spectral region of the isobestic wavelength of hemoglobin, the optical density of the tissue thickness tested by the *in vivo* optical spectroscopy is smaller than 1; therefore it is possible to expand in series the exponential decay of the transmitted light and to obtain, even for continuous-wave dual-wavelength spectroscopy, the simple formula $R_1/R_2 = \mu_{a,1}/\mu_{a,2}$.

On this basis, by properly choosing two commercially available laser diode sources, operating at 750 nm (absorption peak of deoxygenated Hb in the near-infrared) and 810 nm (isobestic point of Hb), a prototype of a non-invasive dual-wavelength continuous-wave oxymeter has been built [3], in which light is guided to and from the sample by means of optical fibers. The expected linearity of the dependence of the ratio R_1/R_2 on the saturation Y, given by

$$R_1/R_2 = \varepsilon_{1,Hb}/\varepsilon_{2,Hb} + Y[\varepsilon_{1,HbO_2} - \varepsilon_{1,Hb}]/\varepsilon_{2,Hb}, \tag{9}$$

has been tested by means of a cow blood circulation simulator, allowing the operator to take blood samples for chemical oxygen saturation analysis and hematocrit value determination. The results are satisfactory and clinical tests on patients under rehabilitation after heart failure are in progress. A limited number of replicas of this prototype will be fabricated, in order to perform more significant clinical tests in different sites.

Acknowledgements

I wish to thank Dr. G. Cicco and Dr. C. Corvasce for useful discussions, and Dr. Ing. P. Lago for his skilful suggestions.

References

[1] E.M. Sevick, B. Chance, J. Leigh, S. Nioka and M. Maris, Quantitation of time- and frequency-resolved optical spectra for the determination of tissue oxygenation, *Anal. Biochem.* **195** (1991), 330–351.
[2] M.S. Patterson, B. Chance and B.C. Wilson, Time-resolved reflectance and transmittance for the non-invasive measurement of optical properties, *J. Appl. Optics* **28** (1989), 2331–2336.
[3] P. Lago, L. Rovati, R. Colombo, U. Corra, F. de Vito and M. Corti, Simple, non-invasive laser diode oxymeter for measurements of human tissues, *SPIE Proc.* **2131** (1994), 475–481.

Role of the endothelium in hypertension: Experimental aspects

V. Vulpis[a], T.M. Seccia[a], F. di Fazio[a], E. Daniele[b] and M.D. Lograno[b]
[a]*DIMO, Division of Internal Medicine, University of Bari, Bari, Italy*
[b]*Department of Farmacobiology, University of Bari, Bari, Italy*

Hemorheological and clotting abnormalities observed in hypertension and atherosclerosis are mostly related to endothelial dysfunction. A reduced vasodilatation in response to acetylcholine (Ach) was found in many experimental models as well as in human hypertension. Stimulation of cholinergic receptors on the endothelium by Ach induces the release of NO that interacts with vascular smooth muscle cells, thus inducing vasodilatation. Depending on the pathogenesis of hypertension, the decreased endothelium-dependent vasodilatation can be caused by either a reduced release of relaxing factors or an increased production of contracting mediators. Indeed, endothelium is a source of a variety of short half-life mediators regulating vascular tone so that an unbalanced production of them could promote or accelerate the hypertensive state. Because of the involvement of these factors in the platelet function as well as migration and proliferation of vascular smooth muscle cells, the abnormal endothelial function can also account for the initiation and development of atherosclerosis. In fact, NO is a strong inhibitor of platelet adhesion and similar to prostacyclin, of platelet aggregation; moreover, aggregating platelets release mediators, such as serotonin, TXA2, ATP, ADP, PDGF and TGF_{β}.

A great amount of data in the literature as well as our experimental results show that endothelial dysfunction in hypertension is a consequence of high blood pressure levels and is related to duration and stage of the vascular disease. Long-term antihypertensive treatment can ameliorate endothelium function in genetic hypertension by reducing blood pressure levels or involving alternative mechanisms. For example, ACE inhibitors can improve endothelial dysfunction by decreasing vasoconstricting agent production as well as modulating the sympathetic nervous system. Our data confirm the beneficial effects on vasorelaxation in spontaneous hypertensive rats. Mechanisms involved in the functional and biochemical changes are different, some of which are presumably inherited as shown by the early abnormalities found at a prehypertensive stage. The decreased vasorelaxant response to Ach observed at this stage can be enhanced by indomethacin in humans as well as in experimental animal models: endothelium-derived contracting factors, and in particular the cyclooxygenase-dependent ones, play a mayor role. A similar effect cannot be reproduced at the advanced stages of the vascular disease.

Taken together, these data suggest that endothelial dysfunction occurs gradually: first, an enhanced production of vasoconstricting factors is associated with a reduced or normal release of vasorelaxant mediators; subsequently structural changes in the endothelial cells induce a decreased NO synthesis and an abnormal response of vascular smooth muscle cells to vasoconstricting agents. The final effects are activation of coagulation mechanisms and hemorheologic abnormalities.

Essential hypertension and platelet aggregability

A. Nardecchia, D. Solazzo and A. Pirrelli

1. Introduction

Platelets can be involved in the development of essential hypertension (EH) as their function is related to pathogenesis and maintenance of the atherosclerotic disease, as they have many properties in common with vascular smooth muscle cells.

Most of the studies point to an activated state of platelets in EH but reports on the platelets aggregation response in EH are conflicting.

The present work was directed to: (1) measuring platelet aggregation response to epinephrine (E) in hypertensive and normotensive subjects, (2) verifying platelet alpha adrenoceptor response in hypertensives by means of two different E concentrations, (3) looking for some correlations among blood pressure, platelets aggregation and cardiovascular risk factors.

2. Materials and methods

We studied 20 patients (age 29–50) affected by essential hypertension and 10 normotensive subjects (age 22–39).

All subjects were in a wash-out period for at least two weeks.

Blood samples were obtained between 8 and 9 a.m. after 14 h overnight fasting. Hematocrit, total cholesterol and fibrinogen were measured in the central laboratory.

Epinephrine (E) and norepinephrine (NE) were dosed by HPLC and electrochemical detector.

3. Platelet aggregation study

Venous blood was collected in plastic tubes containing 3.8% Na citrate with a final volume/volume ratio of 9 : 1 and centrifuged at 250 g for 10 min.

Platelets rich plasma (PRP) was removed and recentrifuged at 4000 g for 20 min yielding platelets poor plasma (PPP). PRP was diluted by PPP to obtain a concentration of 3.0 to 3.5×10^6 platelets/ml. Platelets aggregation was measured photometrically using aggregometer Elvi 840.

E was used as the aggregating agent. The aggregation was studied at different concentrations of E, ranging from 20 to 0.2 μM, evaluated by determining the following parameters: (1) maximum aggregation (AM), expressed as maximum of light transmittance in percentage, (2) slope (S) or maximal aggregation velocity expressed in cm/min in percentage.

4. Statistical analysis

The data of each group of patients (hypertensives and normotensives) were expressed as mean $\pm$ standard deviation. Differences between patient and control groups were analysed using the Student t-test for paired observations ($p < 0.05$). Correlations were studied by linear multiple regression analysis to determine r (the correlation coefficient). Another elaboration of data has been obtained by means of two techniques of multivariate statistical analysis:

(1) analysis of principal components (PCA) to reduce the dimension of the pattern,
(2) methods of multivariant classification (linear discriminant analysis LDA and quadratic discriminant analysis QDA).

5. Results

Platelet aggregating response, expressed as AM and S, showed two different trends in the two groups, depending on the E concentration. At low E concentration AM and S were more elevated in normotensives. At high E concentration AM and S were more elevated in hypertensives and reduced in normotensives. Plasmatic E was more elevated in hypertensives, showing a good correlation coefficient with mean arterial pressure (MAP). None of the other parameters examined showed a correlation with platelet aggregation or MAP.

6. Discussion

We have not shown an enhanced platelet aggregability in hypertensive patients compared with normotensive subjects, but we think to have demonstrated in hypertensives an enhanced sensitivity to high levels of catecholamines determined by the loss of the receptor desensitization capacity. In fact, in normotensives an agonist-induced reduction in platelet α_2 adrenoreceptor affinity for agonist has been identified which is correlated with a fall in receptor sensitivity. This regulation is inefficient, perhaps for genetic transmission, in hypertensives who display an enhanced sympathic tone. The other examined cardiovascular risk factors have played an interesting role in the multivariate statistical analysis.

Video-microscopy of nailfold capillaries in hypertensive subjects

M. Ciccone, M. Lombardi and P. Rizzon
Institute of Cardiovascular Diseases, Bari University, Bari, Italy

1. Introduction

Nailfold capillary microscopy offers a non-invasive method of studying the microcirculation of human skin capillaries and identifying the presence of typical capillary abnormalities in diseases that involve microcirculation disturbances such as hypertension. The aim of this study was to investigate the involvement of skin microcirculation in hypertensives.

2. Method and material

This study was carried out on 10 patients, 3 males, 7 females, age range 52–68 years, with arterial hypertension and 10 patients, 3 males, 7 females, age range 53–70 years as controls.

In all patients we made Video-Capillaroscopy (200×) of the end row capillaries of the nailfold. A drop of cedar-wool oil was applied to the skin to make it transparent. The skin was illuminated, focusing the beam of light of the microscope obliquely on the skin at the base of the fingernail. We identified two different groups:

(a) normal,
(b) abnormal (narrow capillaries, lower mean erythrocyte velocity, sludges).

Table 1

	Normal	Abnormal
Hypertensives	4	6
Control	9	1
p	0.006	

3. Conclusions

In hypertensive patients there is a correlation between blood pressure and the reduction of capillary diameter. In our study we confirm this result, which is a functional expression of increased resistance and lower mean erythrocyte velocity in rest conditions in hypertensives compared with healthy adult subjects.

Blood hyperviscosity and hypertension in the elderly

G. Ranieri, A. Andriani and R. De Cesaris
DIMO, Section of Internal Medicine, Chair of Medical Therapy, University of Bari, Bari, Italy

1. Introduction

Arterial blood hypertension is associated with an increase of blood viscosity through mechanisms that have not been fully elucidated yet. Such an association seems to increase cardiovascular risk.

Many patients with arterial hypertension have abnormal urinary excretion levels of albuminuria, an expression of initial kidney damage.

The aim of this study was to verify the relationship between vascular resistances, blood viscosity and microalbuminuria in elderly hypertensive subjects.

2. Patients and methods

The study was carried out on 70 non-smoker subjects, of both sexes, with normal cholesterolemia levels, aged 69 $\pm$ 4.2 years. Forty were affected by essential hypertension (systolic blood pressure $>$160 mm Hg, diastolic blood pressure $>$95 mm Hg) and 30 were normotensive. Blood samples used to measure whole blood viscosity were obtained from the antecubital vein and treated with dry potassium ethylene-diamine-tetra-acetic acid (EDTA) anticoagulant. Blood viscosity was determined using a couette viscometer with coaxial cylinders (low shear 30 per s). For this study, viscosity was measured at 96 s^{-1}. All measurements were taken at 37°C, achieved by running the viscometer cylinder containing the blood sample through a thermostatically controlled water bath. A pulsed Doppler velocimeter was used to measure the internal diameter (D) and blood velocity (V_m) of the right brachial artery. Both these parameters are essential for blood flow determination ($Q = (D^2 \times \pi/4) \times V_m$). Local resistance was calculated by dividing the mean arterial pressure (MAP) by Q. The microproteinuria assay was carried out on 24-h urines, and repeated for 3 consecutive days with a radioimmunological method. The value considered was the mean of triplicate determinations.

Data are shown as mean $\pm$ standard deviation. Correlation coefficients were calculated using the Pearson product-moment formula. Multiple regression analysis was applied to compare variables independently related to the microalbuminuria.

3. Results

After a 4-week wash-out period, blood pressure in the hypertensive subjects was 187.62 $\pm$ 9.62/ 104.51 $\pm$ 3.95 mm Hg.

Vascular resistances were 144.79 $\pm$ 18.62 mm Hg/ml/s^{-1} in the hypertensives and 128.34 $\pm$ 16.80 mm Hg/ml/s^{-1} in normotensive subjects ($p < 0.00001$). Blood viscosity was higher in hypertensive subjects than in the normotensives (5.43 $\pm$ 0.38 and 4.72 $\pm$ 0.19 mPa s, $p < 0.01$).

Microproteinuria was assayed in the two groups, but was observed only in the 30 hypertensive subjects (171.23 $\pm$ 34.48 mg/dl/24 h) and in none of the normotensive subjects. In the hypertensive group, viscosity compares with SBP ($r = 0.53$, $p < 0.001$) better than DBP ($r = 0.40$, $p < 0.01$). Correlations

were also found between microproteinuria and viscosity ($r = 0.49$, $p < 0.005$), between viscosity and the peripheral vascular resistances ($r = 0.41$, $p < 0.001$) and between peripheral vascular resistances and microproteinuria ($r = 0.49$, $p < 0.005$). In normotensive subjects, no such correlations between investigated parameters were observed.

4. Conclusion

This study shows that in elderly hypertensive patients, but not in normotensive ones, blood viscosity is correlated to vascular resistance, pressure values and microalbuminuria. Multiple regression analysis shows that microalbuminuria is influenced by vascular resistance rather than blood viscosity. Altogether, the above data suggest that increased vascular resistance leads to a change in renal hemodynamics, which is responsible for the increased excretion levels of albuminuria.

Study of the circle of Willis in hypertensive patients by transcranial Echo-Colour Doppler

C. Sabba[a], P. Buonamico[a], D. Damiani[a], C. Di Grassi[a], V. Vulpis[b], A. Pirrelli[b] and O. Albano[a]
[a]*Istituto di Clinica Medica I, Universita' di Bari, Bari, Italy*
[b]*Patologia Medica II, Universita' di Bari, Bari, Italy*

1. Introduction

In the early stage of arterial hypertension there is an increase of cerebral blood flow due to a change in the autoregulation mechanism and, in addition, there is an augmented vasoconstrictive response to acute hypertensive episodes [1]. An acute hypertensive reaction can be provoked even in the early stages of hypertension by various physical or mental stresses designed to increase the adrenergic secretion [2]. The Echo-Colour Doppler technique allows the study of intracranial vessels belonging to the circle of Willis and the mean cerebral artery (MCA) [3]. This Echo-Doppler study was designed to evaluate differences in the distribution of cerebral flow and arteriolar resistances in normotensive subjects with negative family histories of hypertension (N), compared with normotensive subjects with positive family histories of hypertension (P) and patients with systemic hypertension (H).

2. Material and methods

65 subjects were studied: 17 H (mean age 27 ± 9), 25 P (mean age 30 ± 3) and 23 N (mean age 28 ± 6). In all patients MCA was studied by Echo-Doppler (ATL UM9 HDI), choosing the side in which the higher MCA velocity was detectable. In addition, in 27 subjects: 8 H (mean age 32 ± 8), 10 P (mean age 33 ± 4) and 9 N (mean age 29 ± 3), anterior cerebral (ACA), posterior cerebral (PCA) arteries of both sides and the basilar trunk were studied. Pulsatility index (PI) $=$ (peak-minimum frequency)/mean frequency was calculated for all the studied arteries. For MCA, due to a favourable Doppler angle, also maximum (V_{max}), mean (V_{med}) and minimum (V_{min}) velocities were considered. In addition, in this group of patients a hand grip isometric exercise was performed [2]. Systolic (SYS) and diastolic (DIA) blood pressure and heart rate (HR) were monitored all through the experiment, and Doppler measurements on one MCA were performed right before (B) and during the isometric exercise (HG).

3. Results and discussion

In Table 1 the basal values and the results of the hand grip exercise are shown. These data suggest that, in H, the cerebral overflow, indicated by the augment of MCA blood velocity, is not accompanied by changes in distrectual resistance to flow in this vascular territory.

The isometric exercise provoked a significant increased systolic (from 127 ± 3 to 153 ± 5, $p < 0.0001$) and diastolic blood pressure (from 79.1 ± 3 to 96 ± 3.2, $p < 0.0001$) of the cerebral artery.

V_{max} and V_{min} increased from 103.3 ± 3.8 to 113.02 ± 4.8 ($p < 0.01$) and from 51.6 ± 2.2 to 58.8 ± 3 ($p < 0.01$), respectively. However, an increased PI did not accompany this observation.

Table 1

	V_{max}	V_{min}	V_{med}	PI	SYS	DIA	HR
NB	103 ± 8	54 ± 3	70 ± 5	0.71 ± 0.03	118 ± 4	70 ± 4	69 ± 3
NHG	111 ± 9	61 ± 5	72 ± 11	0.72 ± 0.12	135 ± 7	87 ± 6	76 ± 5
PB	100 ± 5	46 ± 4	68 ± 5	0.80 ± 0.05	120 ± 4	75 ± 3	78 ± 4
PHG	111 ± 9	56 ± 6	80 ± 7	0.71 ± 0.07	147 ± 7	92 ± 4	91 ± 5
HB	108 ± 7	55 ± 4	75 ± 5	0.72 ± 0.05	145 ± 3	94 ± 4	74 ± 4
HHG	117 ± 8	60 ± 5	84 ± 6	0.68 ± 0.06	181 ± 6	112 ± 4	87 ± 4

The increase in blood pressure was more evident in H, compared with N and P subjects, which was accompanied by a trend towards an increase in MCA blood velocities quite similar in the three groups, without evident effect on PI, i.e., in MCA territory resistances to flow. As for the other arteries, no significant changes were observed.

If these preliminary data are confirmed by successive studies on a wider population, they might indicate a lack of vasoconstrictive response in normal subjects, with and without family history of hypertension, and borderline hypertensive patients to acute or chronic pressure changes.

References

[1] H.R. Winn, E.C. Haley and R.M. Berne, Cerebral blood flow regulation in normotension and hypertension, in: *Hypertension and the Brain*, G.P. Guthrie and T.A. Kotchen, eds, Futura Publishing Co., Mt Kisco, NY, 1984, pp. 157–189.

[2] B.R. Widgren, J. Wikstrand, G. Berglund and O.K. Andersson, Increased response to physical and mental stress in men with hypertensive parents, *Hypertension* **20** (1992), 606–611.

[3] R. Aaslid, The Doppler principle applied to measurement of blood flow velocity in cerebral arteries, in: *Transcranial Doppler Sonography*, R. Aaslid, ed., Springer, New York, 1986, pp. 22–38.

Transcutaneous oximetry in subjects in hemodialysis

G. Baldassarre[a], G. Cicco[b], G. Passavanti[a], A. Manicone[b], P. Vicenti[b], M. Marra[b], P. Coratelli[a] and A. Pirrelli[b]
[a]*Institute of Nephrology, University of Bari, Bari, Italy*
[b]*Hypertension Centre, University of Bari, Bari, Italy*

1. Introduction and aim

Alterations in microcirculation and tissular oxygenation are often found in nephropatic subjects, especially if they are hypertensives under periodical hemodialysis. During hemodialysis, the dialysis bath (acetate and/or bicarbonate) and blood-membrane lung (coagulation and lympho-monocyte activation) cause hypoxemia. In order to optimise the drug and hemodialysis treatment and to define peripheral perfusion and oxygenation we studied a group of patients in bicarbonate dialysis with mild arterial hypertension, but not undergoing drug therapy, by evaluating transcutaneous oxygen release.

2. Materials and methods

For three consecutive weeks we followed a group of 11 patients (under bicarbonate dialysis three times a week) with mild hypertension controlled by hemodialysis alone.

The patients under dialysis for the same length of time (20 months $\pm$ 4), age (50 $\pm$ 3) and of both sexes (6 male/5 female) were subjected to transcutaneous oximetry (a non-invasive, easily repeatable method) before, during and after hemodialysis treatment. Three transcutaneous oximeters were used contemporarily (one Microgas 7640 MK2 and two Cutan S20 PO_2 Monitor, Kontron Instruments). The $TcPO_2$ measurement sites were standard: subclavian (Combi sensor for PO_2 and PCO_2-Microgas 7640 MK2), right and left calves (Clark Sensor for PO_2 Cutan 879 PO_2). The test conditions were standard with a temperature of 44°C.

3. Results

We noted a slight but significant reduction in the $TcPO_2$ in all tested areas ($TcPO_2$ average decrease $\Delta\% = -8$) ($p < 0.04$) at the end of the first hour of bicarbonate dialysis. This was followed by a significant increase in the remaining hours of dialysis ($TcPO_2$ average increase $\Delta\% = \% + 19$) ($p < 0.01$) with a conclusive increase in oxygen release at the end of dialysis ($TcPO_2$ average increase $\Delta\% = +11$) ($p < 0.03$).

Likewise a direct and significant correlation between the hemocyte base values and those of the $TcPO_2$ at the beginning of dialysis was noted ($p < 0.01$). The traditional hemogas controls confirmed a 90% correspondence of the arterial PaO_2 with the transcutaneous $TcPO_2$.

4. Conclusions

The results of this study suggest that the above-mentioned, non-invasive, easily repeatable method enables the continuous monitoring of the PO_2 variations during dialysis and could therefore be another possible method for studying biocompatibility.

Peripheral vascular reactivity and metabolic consequences in hypertensives

P. Nazzaro, M. Merlo, M. Manzari, R. Triggiani, A.M. Scarano, G. Vapore, G. Ciancio and A.M. Pirrelli
DIMO, Internal Medicine and Hypertension, Stress Research Center, University of Bari, Bari, Italy

The clinical onset of hypertension can functionally be considered as follows. A progression from a state characterised by a central cardiac hemodynamic reactivity with high cardiac output and decreased peripheral vascular resistance into a condition marked by an enhanced vasoconstrictive response with a depressed cardiac output and increased resistance to the peripheral blood flow. The change of the hemodynamic reactivity in the course of hypertension shows that the central nervous system has a substantial role in maintaining the high blood pressure values through the sympathetic nervous system activity. This can respond differently to the environmental and laboratory stimuli elucidating the individual hemodynamic pattern of reactivity, which can demonstrate the sympathetic overdrive.

A sustained neuroadrenergic tone is not only responsible for the functional cardiovascular responses but also for their duration. This was found to distinguish the normotensive individuals with family history of hypertension and those patients who became hypertensive within a short time as well as the borderline hypertensives with a premature onset of a structural damage.

In fact, left ventricular hypertrophy and minimal forearm vascular resistance, related to microvascular angina and to a vascular lumen reduction, were also found in patients with an increased blood pressure variability, such as borderline hypertensives and subjects with “white coat hypertension”. Characteristically, in these patients a sympathetic overreactivity is often found and the structural damage was related to the trophic effects of the neuroadrenergic activity.

Different studies also related the enhanced sympathetic tone to various disturbances in metabolisms frequently observed in patients with high blood pressure values, such as hypercholesterolemia and hyperinsulinemia.

In a previous study, we found that the reduction of hypercholesterolemia per se reduced the vascular damage and the regional hemodynamic reactivity to laboratory tasks in mild hypertensives who were not treated for their blood pressure. On the other hand, the antihypertension treatment, with a certain efficacy in reducing the sympathetic peripheral activity, had a slight but significant effect in lowering the dyslipidemia in these patients when only the hypertension was treated. The findings seem to suggest that hypertension, cholesterol level and peripheral hemodynamic responses can be associated with the sympathetic nervous system arousal.

Recently we performed a study to investigate the relationship between insulin resistance and high blood pressure which both frequently affect the same patient. Obese normotensives and lean very mild hypertensive patients, both with a significant insulin resistance, were compared with lean insulin-sensitive normotensives. We studied the muscular circulation because the insulin activity is particularly crucial in this tissue. Although the vascular damage index did not differ among the subjects, only the hypertensives showed a regional vasoconstrictive stress response. The results demonstrate that an unbalanced peripheral vascular response, already found in the pre-clinic phases of primary hypertension, could be associated with the onset of insulin resistance. This might be, therefore, secondary to the peripheral vascular dysreactivity and the restrained muscular blood flow. Alternatively, the explanations for the origin of the insulin resistance state may vary in different patients with and without essential hypertension.

The relationship between the peripheral vascular hemodynamic disturbances and the state of reduced insulin sensitivity may also be highlighted by studies on the microcirculation. In fact, intravital video-

Table 1

	$GLUC_{auc}$	INS_{auc}	SBP	DBP	HR
Hi-NT	12352 ± 411	$13421 \pm 1693^{*}$	$128 \pm 4^{*,**}$	$82 \pm 1^{*,**}$	67 ± 3
Hi-BHT	14617 ± 973	$13733 \pm 1629^{*}$	148 ± 5	94 ± 3	71 ± 1
Ni-BHT	13755 ± 527	7752 ± 444	146 ± 4	92 ± 3	72 ± 1

Data as mean $\pm$ SE; $^{*}p < 0.05$ vs. Ni-HT; $^{**}p < 0.05$ vs. Hi-HT; $^{***}p < 0.05$ vs. Hi-NT.

Table 2

	$FABF_{aucR}$	$FAVR_{aucR}$	LDF_{aucR}	mFAVR	$LDF_{postoc.}$
Hi-NT	21.2 ± 5.1	-205.7 ± 109.1	45.67 ± 21.2	2.1 ± 0.1	36.2 ± 10.2
Hi-BHT	$11.4 \pm 4.4^{***}$	$-14.7 \pm 48.7^{***}$	$-47.5 \pm 31.2^{***}$	$3.4 \pm 0.3^{***}$	$21.1 \pm 9.2^{***}$
Ni-BHT	$12.8 \pm 4.6^{***}$	$-89.2 \pm 108.3^{***}$	$-25.4 \pm 20.7^{***}$	$3.1 \pm 0.2^{***}$	$26.8 \pm 8.8^{***}$

capillaroscopic techniques pointed out that both hypertensive patients and a reduced capillary density may affect subjects with non-insulin-dependent diabetes mellitus.

Purpose of our study was to identify the macro- and micro-vascular stress reactivity in insulin resistant borderline hypertensive patients. To recognize the impact of the early stages of primary hypertension on the vascular disorders we compared hyperinsulinemic normotensives (Hi-NT) and borderline hypertensives (Hi-BHT) with normoinsulinemic hypertensive patients (Ni-BHT). All the subjects were males and matched for age, smoke habit and history of hypertension. The insulin sensitivity state was ascertained calculating the total functional response of insulin (INS_{auc}) during a two-hour oral glucose tolerance test ($GLUC_{auc}$) (cf. Table 1).

Through a sylastic strain-gauge plethysmograph we measured the regional blood flow (FABF) and resistance (FAVR), while the total superficial microvascular flow was taken by a laser-Doppler probe placed at the forearm (LDF). The patients underwent psychological (Color Word Stroop) and physical (Forehead Cold Pressor Test) stimuli. The reactivity was evaluated calculating the "area-under-the-curve" (value $\times$ time) as total dynamic response ($\text{aucR} = \text{aucTOT} - \text{aucBSL}$).

Postischemic maximum forearm blood flow was calculated and the residual vascular resistance (mFAVR) and the postocclusive laser-Doppler fluxmetry percentile change ($LDF_{postoc.}$) served, respectively, as index of vascular structural damage and microvascular filling properties (cf. Table 2).

These preliminary results demonstrate that hypertensive patients with and without hyperinsulinemia showed a reduced regional vasodilating stress response, which was unlike that in normotensive insulin-resistant subjects. Also, the microcirculation demonstrated a more pronounced vasoconstrictive response in patients with high blood pressure. On the other hand, vascular damage and reduced microvascular hemodynamic properties would seem to indicate hypertension in its early stages and might explain the unbalanced glucose dysmetabolism in patients with hypertension.

The effect of controlled physical activity on peripheral perfusion in normotensives with a family history of hypertension

E. Dolce[a], G. Cicco[a], A. Manicone[a], A. Scardicchio[b], M. Marra[a] and S. Cesario[b]
[a]*DIMO, Internal Medicine and Hypertension, University of Bari, Bari, Italy*
[b]*Sportive Medicine Centre, Policlinico, Bari, Italy*

1. Introduction

Physical exercise requires greater oxygen availability in the muscles. This oxygen is supplied in two ways: extraction from the tissue and/or an increase in muscular blood flow. The latter is possible due to a series of cardiocirculatory adjustments, which are more or less the same for both sedentary subjects and trained athletes. A modification of the systemic arterial blood pressure is one of these cardiocirculatory adjustments, therefore post-exercise hypertension and its duration is of great relevance. In fact, the ergometric test is often used to measure the risk of hypertension. The strategy of comparing normotensives with or without a family history of hypertension has been widely used in the last few years. This strategy enables the evaluation of the possible etiological role of various factors such as vascular reactivity, sodium uptake and transport, baroreceptor sensibility and the intervention of the autonomous nervous system, which bring about the development of hypertension. The aim of the study was to evaluate the variations in peripheral perfusion using transcutaneous oxymetry after physical exercise and to find possible correlations with arterial blood pressure values in patients genetically predisposed to hypertension.

2. Materials and methods

We studied 35 young male subjects following the Helsinki agreement of 1975. The patients had the following characteristics in common:

(1) age, daily habits;
(2) regular higher than normal blood pressure values;
(3) absence/presence of a family history of hypertension (the latter valid for both parents);
(4) willingness to carry out aerobic exercise for 45 min three times a week for a period of 10 weeks.

Before and after this 10-week period we measured the peripheral perfusion via transcutaneous oxymetry and calculation of the Regional Perfusion Index (RPI). We also measured the systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) values. These measurements were taken in baseline conditions and after the Head Up Tilt Test at 3, 5, 7, 10 and 15 min after the execution of the test.

After a period of rest the patients undertook the ergometric cycle test which, using Finapress, automatically registered blood pressure (BP) and heart rate (HR) at preset levels. After the test we measured the SBP, DBP and HR after 3, 5, 7, 10 and 15 min rest. We also evaluated the correlation between the SBP during rest and the SBP at the peak of exercise.

Table 1
SBP ratio before and after training period in patients with a family history of hypertension

Recovery time	SBP ratio before study	SBP ratio after 10 weeks	p value
R_3	0.84 ± 0.09	0.83 ± 0.08	n.s.
R_5	0.75 ± 0.10	0.78 ± 0.09	n.s.
R_7	0.69 ± 0.09	0.64 ± 0.05	<0.001
R_{10}	0.64 ± 0.06	0.63 ± 0.07	n.s.
R_{15}	0.60 ± 0.08	0.58 ± 0.06	n.s.

Table 2
RPI before and after training period in subjects with a family history of hypertension

Recovery time	RPI before study	RPI after 10 weeks	p value
R_3	0.68 ± 0.16	0.72 ± 0.12	n.s.
R_5	0.70 ± 0.17	0.76 ± 0.16	n.s.
R_7	0.80 ± 0.08	0.87 ± 0.11	<0.005
R_{10}	0.72 ± 0.13	0.74 ± 0.09	n.s.
R_{15}	0.60 ± 0.14	0.74 ± 0.08	n.s.

3. Results

In subjects with a family history of hypertension there was a significant difference in the RPI ($p < 0.001$) 7 min after both tests. The other parameters showed no significant change.

The results (Tables 1 and 2) suggest an adjustment of both the blood pressure and peripheral perfusion values in subjects with a family history of hypertension, which could be one of the many genetic explanations for hypertension. As a matter of fact, recent studies seem to indicate that there is a genetic preselection for performance at the height of exercise and that the endothelium-dependent muscular relaxation is increased by chronic exercise. Physical exercise also presupposes an adaptation of the sympathetic system (specifically studied using the Head Up Tilt Test).

4. Conclusion

Subjects with a family history of hypertension showed after 7 min of testing and 10 weeks of study, a significant decrease in recovery time ($\Delta\% = -7$, $p < 0.001$) and also a significant increase in RPI ($\Delta\% = +9$, $p < 0.005$). These results could be related to a vasodilatation in microcirculation and an improvement in peripheral perfusion and oxygenation after a training period.

Transcutaneous oxymetry in a patient with HBC/β^0 thalassaemia and non-insulin-dependent diabetes

P. Izzo[a], A. Manicone[a], A. Spagnuolo[a], G. Cicco[b] and A. Pirrelli[b]
[a]*DIMO, Biomedical Sciences and Human Oncology, University of Bari, Bari, Italy*
[b]*DIMO, Internal Medicine and Hypertension,University of Bari, Bari, Italy*

1. Introduction

The oxyhemoglobin dissociation curve and the peripheral factors, which affect it, influence oxygen delivery to the tissues. Red blood cells containing hemoglobin C show rheological abnormalities, which depend on the crystallisation of the hemoglobin, during the oxyhemoglobin state, and on the hypertonic environment. Non-insulin-dependent diabetics could find themselves in a compromising condition due to the hemodynamics of peripheral oxygenation.

2. Materials and methods

We measured oxygen delivery to the tissues in a 66-year old patient with HbC/β^0 thalassaemia and non-insulin-dependent diabetes. The patient also suffered from mild anaemia (Hb 10 g%, Hematocrit 33%, indirect bilirubin 3.7 mg%) and during hemoglobin electrophoresis on gelatinised cellulose acetate and electrophoresis of the polypeptide chain, was found to have only α and β^c fractions. The 2,3-DPG was measured using the Boehringer-Mannheim method.

For the study we used 3 Oxymeter simultaneously: in the subclavian area (Microgas 7640 MK_2, Kontron Instruments, with a Combi sensor) to measure the pO_2 and pCO_2 and in the ankle (820 Monitor, Kontron Instruments, with a Clark sensor) to measure the pO_2. The sensors were kept at a constant temperature of 44°C. We calculated the RPI (the ratio between the $TcPO_2$ at the calf and the $TcPO_2$ at the subclavian area). The Doppler was used to measure the Winsor Index (Ankle Brachial Pressure Index) in order to exclude possible alterations in peripheral arterial circulation. The peripheral blood flow was also measured using the laser-Doppler technique (Oxford Optronix Oxford Array).

3. Results

In the present case, we observed a reduction in peripheral oxygen release with the RPI lowered to 0.36 (see Table 1). The 2,3-DPG was reduced by 50% even though the patient was anaemic. This reduction caused a shift to the left of the oxyhemoglobin dissociation curve with a consequent increased affinity for oxygen and a reduction in its peripheral release.

The post-ischemia laser-Doppler study showed a 20% reduction of the blood flow rate in the arm, whereas the highest post-ischemia vascular flow rate was decreased. The minimum post-ischemia resistance, which is an indicator of alterations in vasoconstriction, was slightly increased. These hemodynamic alterations in the blood flow are symptoms of non-insulin-dependent diabetes.

Table 1

Hemoglobin	9.9 g%
Hematocrit	33%
Reticulocytes	1.78%
Glycemia	141 mg%
2,3-DPG	2.5 mmol/l
$TcpO_2$ subclavicular	68 mm Hg ↓
RPI	0.36 ↓
Δ% LD finger post	69.51 ↓
Δ% LD arm post	22.23 ↓
Maximum forearm bloodflow	33.18 ↓
Minimum forearm vascular resistance	3.23 ↑

4. Conclusions

The reduction in peripheral oxygenation is a result of the reduction in peripheral blood flow which could be due to the rarefaction of the vascular network (which was demonstrated by capillaroscopic studies) and/or due to the reduction in peripheral perfusion as demonstrated by the laser-Doppler and plethysmographic techniques. All this is typical of non-insulin-dependent diabetes.

The reduction in 2,3-DPG, as shown by other studies, does not seem to be the result of hemoglobin pathology, but could depend, in this case, on its reduced synthesis. The latter may be caused by a block in the glycolytic pathway in the Rappaport–Luebering cycle or by a reduced affinity for oxygen found in the hemoglobin C in living subjects.