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Thoughts and Progress

Oxygenation–Ozonation of Blood During Extracorporeal Circulation: In Vitro Efficiency of a New Gas Exchange Device

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Abstract: We have investigated the performance of a new gas exchange device (GED), named L001, specifically devised for the ozonation of human blood during extracorporeal circulation. This procedure, defined with the acronym "EBOO," means "extracorporeal blood oxygenation–ozonation." The innovative GED is made of microporous, ozone-resistant, polipropylene hollow fibers with an external diameter of 200 μm, a thickness of 50 μm, and a membrane surface area of 0.22 m^2. The material is coated with phosphorylcholine on the external side in contact with the circulating blood, while a gas mixture, necessarily composed of medical oxygen and ozone (about 99 and 1%, respectively), flows inside the fibers in opposite direction. The new GED has been tested by using a buffered saline solution containing KI and by varying several parameters, and it has shown to be very versatile and efficient. Its main characteristics are minimal foreign surface contact, high gas transfer, and negligible priming volume. This device appears to be a practical, nontoxic, and rather inexpensive tool for performing ozonation of blood for already defined human diseases. **Key Words:** Ozone—Extracorporeal circulation—Polipropylene hollow fibers—Phosphorylcholine coating—Gas exchange device.

The idea to realize a dialysis-like system for a mild blood ozonation of blood ex vivo was fairly common in the 90s (1), and unfortunately, even today, dialysis filters are used in private clinics for cancer and human immunodeficiency virus-infected patients hoping for a cure. Dialysis filters are made with hydrophilic hollow fibers to allow passage of water and solutes into the dialysate but are inefficient and not idoneous for gas exchange. Moreover, being made of ozone-sensitive materials, they can release toxic compounds in the circulating blood. Thus, only gas exchange devices (GEDs) made with hydrophobic, water-impermeable, ozone-resistant, polypropylene hollow fibers enclosed in an ozone-resistant housing and connected to the peristaltic pump and ozone generator by silicone tubing must be used. During the last 8 years, we have performed a number of investigations aiming to establish a new method for the oxygenation–ozonation of human heparinized blood during extracorporeal circulation using GEDs, namely, oxygenators, typically used in open heart surgery. We are indeed interested in not simply oxygenating blood because, as explained in the Discussion, our aim was to deliver minimal doses of ozone, which, at appropriate concentrations, can activate a number of biological functions in blood cells without any deleterious effect. In the first article, we have evaluated the methodology and performed a preclinical evaluation in sheep (2), while initial clinical studies have examined the efficacy of the system in a variety of vascular diseases (3) and in one patient with necrotizing fasciitis (4). In contrast to dialysis filters, the GED allows blood to flow outside the hollow fibers while the gas mixture (~99% medical oxygen and ~1% ozone) flows in a counter-current fashion inside the hollow fibers, but we noted some problems related to the scarce bioavailability of the devices. We have also tested GEDs with fibers externally coated with either heparin or human albumin (5,6) but, although albumin reduces platelet adhesion better than heparin, GEDs are not satisfactory. This is so because the strong ozone reactivity stimulates the aggregation of platelets that, in spite of the presence of heparin, adhere onto the external surface of the fibers and cause a progressive reduction of the gas transfer and the GED's efficiency. We have already extensively discussed the problem why, in the presence of ozone, neither heparin nor albumin represents the ideal biocompatible coating for a foreign surface (6). However, the last clinical study (7) has been performed with GEDs coated with phosphorylcholine, which represents a great advance in terms of biocompatibility (8). The present article aims to describe a new GED, specifically engineered for the EBOO. This device tries to optimize the process of oxygenation–ozonation of blood.

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MATERIALS AND METHODS

In this study, we examined in detail a new GED, model L001: the membrane, 50 μm thick, was made of microporous polypropylene, with a gas exchange surface area of 0.22 m^2 coated with phosphorylcoline, lined on the surface in contact with blood to prevent adhesion of platelets and procoagulant proteins. The hollow fibers had an external diameter of 200 μm and an internal one of 150 μm. The device weighed 236.0 g; the maximal blood flow was of 0.7 L/min, and the priming volume was as little as 20 mL. It was produced by SORIN Group Italia, Mirandola (Modena, Italy), patent pending. Figure 1a,b shows the external form and a section, respectively. Figure 1c describes in detail the various parts. We made a partial comparison with the older model L2, which had a surface of 0.65 m^2 (patent no. 0582959) used in the clinical trial published in 2005 (7).

The GED and lines were routinely rinsed with 2-L saline before starting the perfusion. Ozone was produced by three generators by using only pure, medical oxygen constantly delivered at no more than 1.5 bar:

1. Ozonline ECO_3 (Torino, Italy). It delivers ozone concentrations between 0.1 and 3.0 μg/mL of gas, with a gas flow ranging from 250 mL/min (15 L/h) up to 1 L/min (60 L/h).
2. Ozonline. It delivers ozone concentrations between 1 and 15 μg/mL of gas with a gas flow ranging from 1.60 and 6.0 L/min.
3. Ozonosan PM-80 (Hansler GmbH, Iffezheim, Germany), which could deliver ozone concentrations from 2 up to 80 μg/mL with a gas flow ranging between 1 and 8 L/min.

We felt compelled to use these three ozone generators because we needed to test a wide range of ozone concentrations and, to date, a generator able to deliver these concentrations is not yet available.

Ozone was generated from oxygen using electrical corona arc discharge. Silicone and, when necessary, Tygon polymer tubings (Saint-Gobain Performance Plastics, Lyon, France) were used throughout the reaction procedure to ensure containment of ozone and consistency in concentrations. The unused ozone was immediately converted to oxygen by passing through a destructor (Fig. 1c).

Within the ranges of ozone concentrations indicated earlier, medical oxygen represented between 95.0 and 99.8% of the gas mixture, while ozone was the residual percentage. In all cases, the ozone concentration was monitored continuously by photometry at 600 nm (Chappuis band), periodically checked by iodometric titration. GEDs and ancillary materials used in this investigation were sterile and used only once. The precise entity of the gas flow was periodically checked with a Rota Yokogawa, Typ: RAGL 53 (Wehr, Germany).

Most of the chemical determinations were carried out in the Physiology and Pharmaceutical Chemistry Laboratories of the University of Siena, while those performed with the ozone generator B were carried out in the Dialysis Unit of the University Polyclinic, Siena, Italy. The protocol (no. 562/05, June 30, 2005) was approved (February 28, 2006) by the Ethical Committee of the University of Siena.

The solution used throughout the experiments simulated deproteinized serum and consisted of a freshly prepared saline (0.145 M), phosphate buffered (0.05 M) solution adjusted at pH 7.3. KI (0.12 M) was added just before the experiments, and the final solution was clear and colorless.

The efficiency of the GEDs to transfer ozone flowing inside the hollow fibers to this solution, flowing in a counter-current fashion outside the hollow fibers via a precise peristaltic pump (Multimat B, Bellco, Modena, Italy), was determined by the iodometric method according to the revised procedure described by Masschelein (9). All samples were collected in 50-mL sterile polypropylene test tubes immediately closed with Teflon caps (VWR International S.r.l., Milan, Italy). No recirculation of the solution was allowed because it was discarded after sampling. The iodometric reaction was carried out immediately at the end of the experiment. When ozone reacts with the KI solution, iodine is generated and the solution immediately acquires an amber color which, upon reduction with a titrated solution of $Na_2S_2O_3$ and a starch indicator allows the determination of the ozone concentration:

$$O_3 + 2I^- + H_2O \rightarrow I_2 + O_2 + 2OH^-$$

$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$$

The concentration of ozone in gram per liter equals 24 × volume of thiosulphate in L × normality of thiosulphate divided by the inlet volume of gas flow in liters. Both the entities of the gas and of the solution volumes per min were precisely determined at 21°C and normal atmospheric pressure.

The detection limit of the analytical procedure was 0.1 mg/L, and the reproducibility was ±2% of the measured ozone concentration. All determinations were repeated at least three times and proved to be very reproducible (coefficient of variation <2%). Figures 2–5 present the mean value.

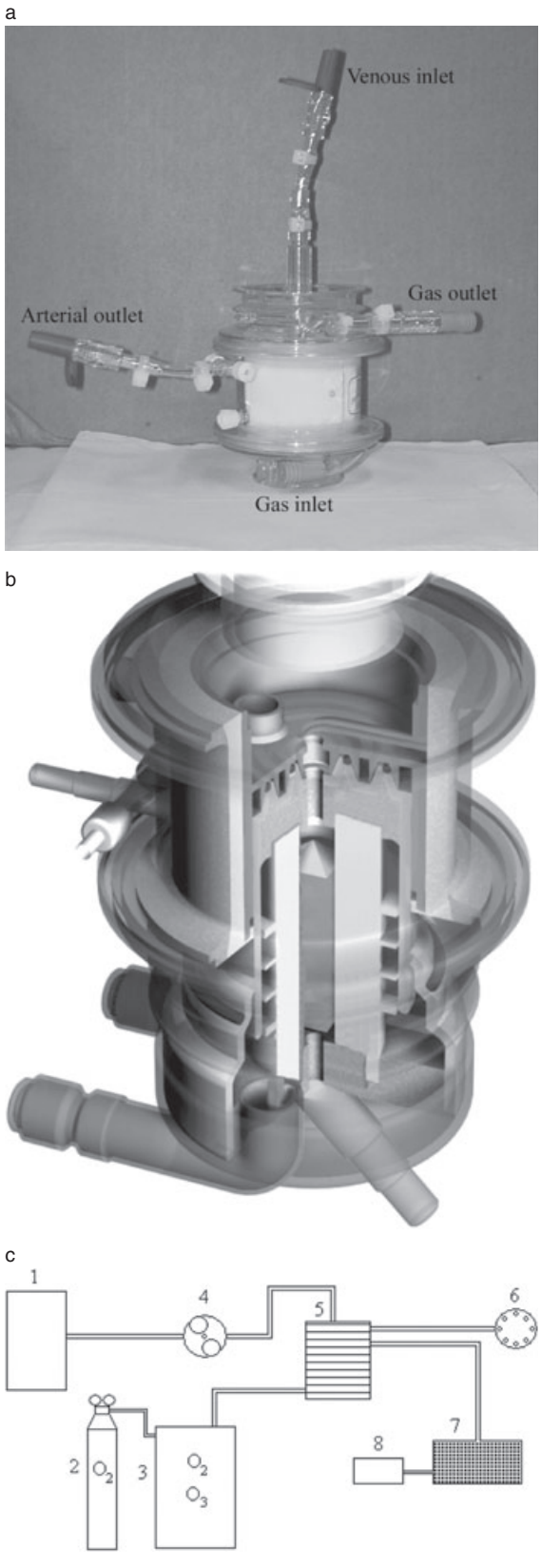

FIG. 1. GED L001 (a) the external aspect, (b) the cross section, and (c) a schematic diagram of the circuit (1, saline solution; 2, oxygen supply; 3, ozone generator with photometer; 4, peristaltic pump; 5, GED; 6, sample collector; 7, silica gel trap; 8, ozone destructor).

RESULTS

Owing to the fact that ozone is a strong oxidant, it must be used with great caution and we must gain a precise knowledge of the efficiency of the GED. For these reasons, we ran the first series of experiments (by using ozone generator A) to evaluate the amount of iodine formation with a constant flow of the saline–KI solution of 80 mL/min, by using very low ozone concentrations (from 0 to 2.4 μg/mL of gas) delivered at a constant gas flow of 250 mL/min. Consequently, the ozone doses range between 0 and 0.6 mg/min. Figure 2a shows the kinetic of the trans-

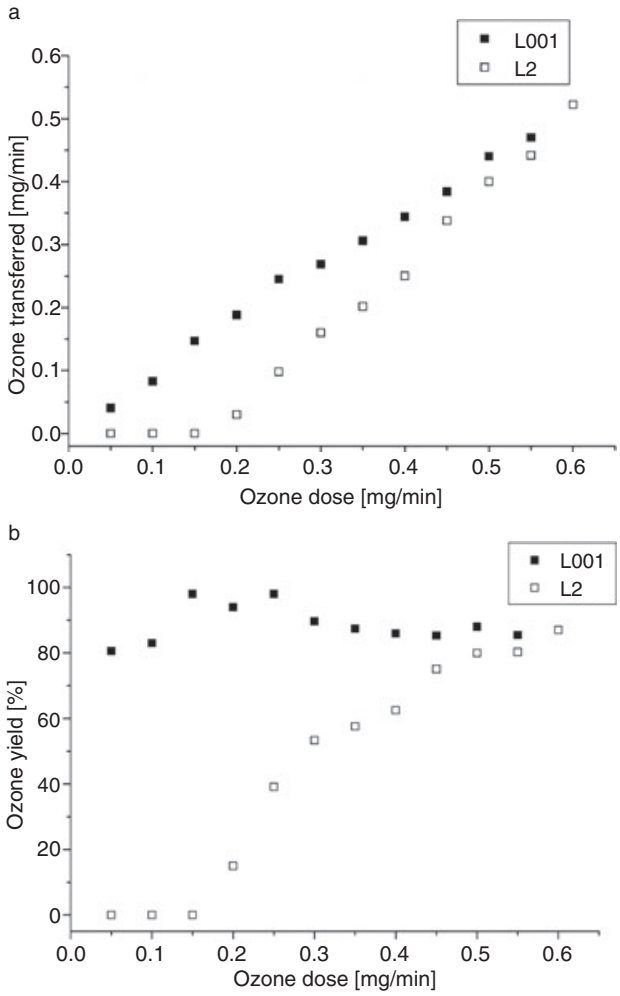

FIG. 2. (a) Transfer and (b) total yield of ozone obtained by using ozone generator A and two different GED sizes.

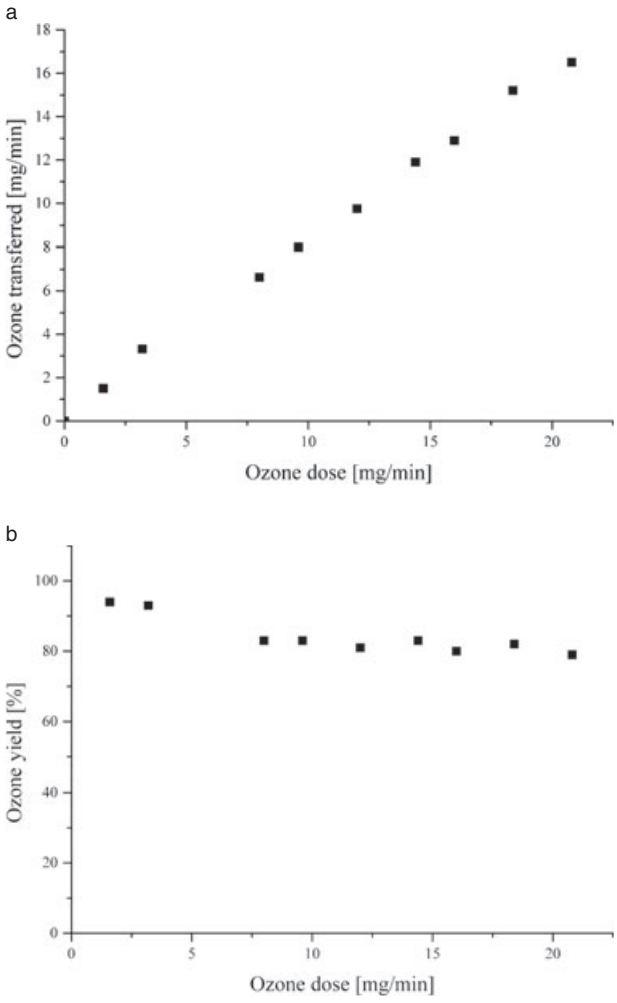

FIG. 3. (a) Transfer and (b) total yield of ozone by using GED L001 and the ozone generator B.

fer of ozone; hence, the iodine formation increased in a linear fashion for the GED L001, while the L2 was defective at the initial ozone doses. Figure 2b demonstrates that the net yield of ozone, expressed as a percentage, is almost quantitative (80–98%) for the model L001.

Next, with the more powerful generator B, we thought that it was worthwhile to investigate the effect of higher ozone concentrations, from 1 to 13 μg/mL of gas delivered at a constant gas flow of 1.60 L/min, and therefore ozone doses ranging between 0 and 20.8 mg/min. The saline–KI solution was pumped constantly at the usual flow of 80 mL/min. Figure 3a shows the linearity of the ozone transferred from the gas phase flowing inside the hollow fibers and the external iodide solution. Figure 3b shows that the ozone yield ranges between 80 and 94%.

During open heart surgery, blood needs to be oxygenated with far higher volumes of the gas mixture (from 1 to 4.5 L/min) and therefore, by using the ozone generator C, we examined the L001 behavior undergoing these gas volumes, with ozone concentrations ranging from 4 to 69 μg/mL and therefore with ozone doses ranging between 15 and 124 mg/min. Figure 4a shows that, in spite of a wide change of gas flow, the amount of ozone interacting with the transferred KI remains fairly constant. However, Fig. 4b shows that the ozone yield is higher for the gas flow of 1 L/min (between 93 and 88%) and decreases from 76 to 65% for a gas flow of 2.5 L/min and from 83 to 60% for the highest flow (4 L/min). These results were somewhat expected because, in a preliminary experiment, we noted that the progressive increase of the ozone concentration and gas flow allowed only a partial transfer of ozone from the gas to the liquid compartment: in fact, an increased amount of ozone could be recovered in a suitable iodide trap posed after the GED. Nonetheless, these data might be useful later on, if we need to perform oxygenation–ozonation of blood during heart surgery.

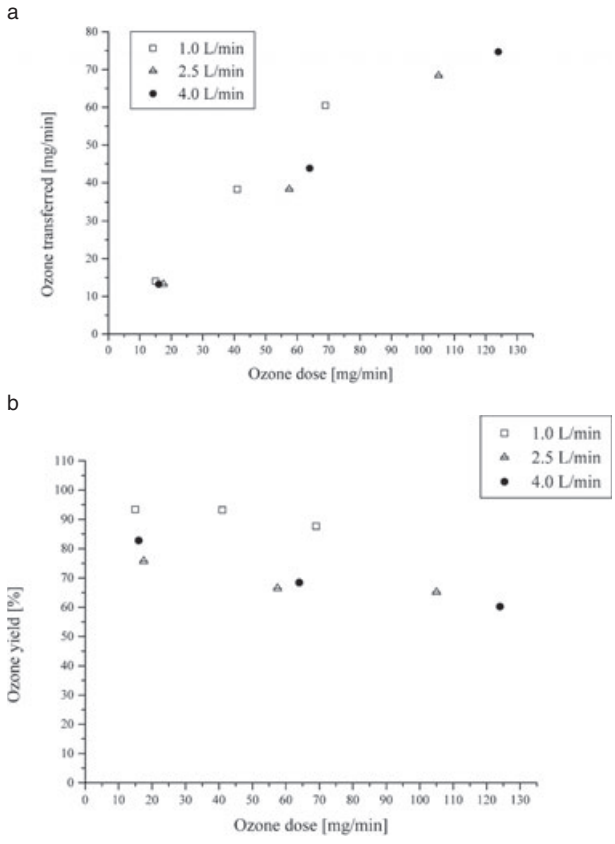

FIG. 4. (a) Transfer and (b) total yield of ozone by using GED L001 and the ozone generator C.

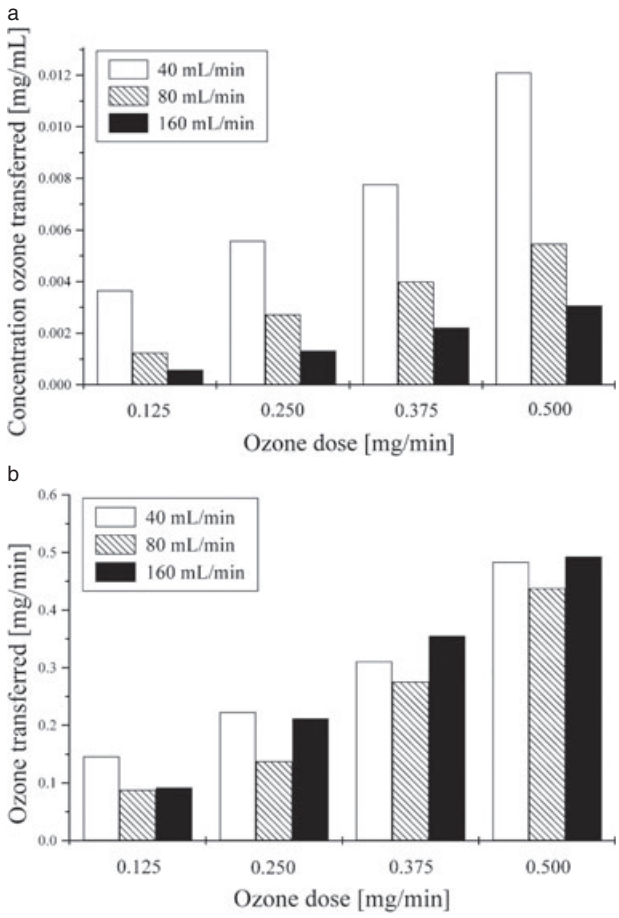

FIG. 5. (a) Transfer and (b) total yield of ozone by using GED L001, the ozone generator A, and three different flows of the buffered saline–KI.

Another critical factor might be represented by the individual caliber's variation of the venous access, which might limit or enhance the volume of blood ozonated per minute. Thus, by using the ozone generator A, we examined four ozone doses (0.125, 0.250, 0.375, and 0.500 mg/min) reacting with the saline–KI solution by testing the model L001 with three different saline–KI flows, namely 40, 80 (as the usual volume), and 160 mL/min. With this system, even by using 16G needles, the patient could not tolerate the withdrawal and return of more than 160 mL/min blood. In all cases, the gas volume delivered per min was of 250 mL (15 L/h). As could be expected, Fig. 5a shows that the amount of ozone reacting with the iodide is inversely proportional to the volume of the saline–KI solution pumped per minute in the GED L001. Moreover, Fig. 5b shows that the total amount of ozone reacted is about constant in relation to the three different saline–KI volumes delivered per min, but, obviously, it increases in relation to the four ozone doses. It appears superfluous to report the diagram showing that in all cases, the percentage of reacted ozone varied between 80 and 98%. On the basis of these results, Table 1 exemplifies how, after having selected the most suitable ozone concentration and dose for the patient, by using the L001 GED, we can define the perfusion time of about an hour.

DISCUSSION

The objective of this study was to evaluate the efficiency of a new GED specifically designed for the ozonation of blood during extracorporeal circulation. Although the total surface of gas exchange has been reduced to only 0.22 m^2, the results obtained, varying several parameters such as ozone concentration, gas volume, and liquid volume, have emphasized the versatility and efficiency of the L001 model. The unique scope of the GED is to allow a mild and well-checked ozonation of human blood during a therapeutic session of about 60 min. Although the gas mixture is usually composed of almost 99% medical oxygen, the residual 1% ozone is quite sufficient for achieving the

TABLE 1. *Definition of perfusion time by using different ozone concentrations and gas volumes*

Ozone (mg/L) Gas flow (15 L/h)	Ozone dose (mg/min)	Ozone transferred (mg/min)	Time of treatment for a 5-mg dose (min)	Time of treatment for a 10-mg dose (min)	Time of treatment for a 15-mg dose (min)	Time of treatment for a 20-mg dose (min)
0.2	0.05	0.0403	124	248	372	496
0.4	0.10	0.0830	60	143	214	285
0.6	0.15	0.1469	34	68	102	136
0.8	0.20	0.1882	27	53	80	106
1.0	0.25	0.2448	20	41	61	82
1.2	0.30	0.2688	19	37	56	74
1.4	0.35	0.3062	16	33	49	65
1.6	0.40	0.3437	15	29	44	58
1.8	0.45	0.3840	13	26	39	52
2.0	0.50	0.4397	11	23	34	45
2.2	0.55	0.4704	11	21	32	43

desired blood activation (10). This is accomplished because ozone dissolves readily in the water of plasma and immediately reacts with hydrophilic reductans and polyunsaturated fatty acids mostly bound to albumin. The reaction generates a cascade of compounds such as hydrogen peroxide and lipoperoxides that (i) improve blood circulation and oxygen delivery to ischemic tissues; (ii) improve the general metabolism; (iii) induce a mild activation of the immune system; (iv) correct a chronic oxidative stress by upregulating the antioxidant system; (v) procure a state of well-being in the majority of patients by activating the neuroendocrine system, and (vi) do not cause any side effects. All of these aspects have been extensively discussed in previous publications (10,11). It goes without saying that blood emerging from the GED is hyperoxygenated (the pO_2 has a tension of about 400 mm Hg), but this aspect is practically irrelevant because the normally used blood flow of 80 mL/min mixes with about 5 L of venous blood before reaching the lungs. However, the new L001 GED can allow, if needed, a blood flow of 700 mL/min, but, obviously, in such a case, we should need to use the ozone generator A, able to deliver ozone at a concentration as low as 0.1 μg/mL.

The small size of the new GED is particularly valid because of the negligible priming volume and the absence of a thermostatically controlled heating system, truly unnecessary. Thus, it is very simple and practical to operate; it is less expensive than previous models; it avoids blood loss, and, being ozone resistant, it does not release foreign compounds in the circulating blood. Having previously used the L2 GED (7), we have already clarified that, in comparison to heparin and albumin, the phosphorylcholine coating on the external surface of the GED in contact with blood is exceptionally biocompatible (8) and avoids the generation of complement complex and the activation of procoagulant factors. Neutrophils, monocytes, and particularly platelets are exquisitively sensitive to ozone (12), but the phosphorylcoline coating and the low ozone concentration avoid the adhesion and the activation of these cells.

Previous clinical experience has already clarified the breadth of therapeutic efficacy of this system in infectious, metabolic, and vascular diseases (3,4,7,11).

Work in progress aims to further clarify a number of biochemical modifications, namely, variation of the blood antioxidant capacity, lipoperoxide concentration, thiol oxidation, hydroperoxide levels, free haemoglobin, and lactic dehydrogenase. The results will be reported in the second part of this series.

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