

Nitric Oxide: Platelet Protectant Properties During Cardiopulmonary Bypass/ECMO

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Abstract: Postoperative bleeding is a major complication in patients who have been placed on extracorporeal circulatory support for various cardiac procedures (1). The increase in hemorrhage is well documented and is associated with various factors, which include, high-dose systemic heparinization, thrombocytopenia, and impaired platelet function. Platelets activate when exposed to the large foreign surface of the extracorporeal circuit, with the largest area being the oxygenator. Despite adequate heparinization, platelet levels continue to decrease. This aggregation phenomenon has also been extensively studied, and it cannot be attributed to the use of aminocarproic acid, aprotinin,

propofol, or amicar (2). Other factors found to be unrelated include, the brand or type of oxygenator, the use of heparin coatings (3), activated clotting time (ACT) levels while on bypass, the operative procedure, preoperative medications, or the types of anesthetic agents used (2). Therefore, it may be beneficial to add nitric oxide to the sweep gas to decrease platelet loss, platelet damage, postoperative bleeding, and lessening the need for post-operative blood transfusions. **Keywords:** cardiopulmonary bypass, ECMO, nitric acid, platelets, post-op hemorrhage. *JECT. 2002;34:144-147*

Platelets, also referred to as thrombocytes, are important cells in hemostasis and blood coagulation. They are produced by the fragmentation of the megakaryocyte, which is derived from the uncommitted stem cell that is produced in the bone marrow. Platelets are included in the category of formed elements of the blood; the other formed elements include the red and white cells. These formed elements make up 45% of the total blood volume. The remaining 55% of the total blood volume consists of plasma (4). Platelets are small, colorless, irregularly shaped fragments, which are 2 to 4 microns in diameter and have a 7- to 10-day life span (4). The normal platelet counts for adults range from 150,000 to 350,000 per mm². The physical properties of platelets play a vital role in the clotting cascade. These properties include, aggregation, agglutination, and adhesiveness (4). For adequate coagulation to occur, platelets must be adequate in number and function. When damage to the endothelium occurs, platelets adhere to the vessel wall and begin to aggregate, or collect, to form a platelet plug (4). This same reaction occurs when platelets are exposed to foreign surfaces (such as the cardiopulmonary bypass circuit). Platelets normally circulate freely in the plasma, but when exposed to damaged epithelium or a foreign surface, they begin to

activate. When platelets are activated, there is a drastic change in their morphology. Before activation, platelets are smooth, irregularly shaped fragments that lack a nucleus. Once activated by endothelial damage or foreign surface exposure, they transform and release potent biochemicals (such as thromboxane A₂), which causes further aggregation (4). The platelet plug becomes stabilized by the activation of thrombin and fibrin (4). Platelets are extremely adhesive to each other, as soon as blood is removed from a vessel they adhere to themselves and every other foreign surface they come in contact with. These factors help to explain the reason behind large amounts of platelet consumption that occurs in the oxygenator. The excess of platelet activation occurring in the oxygenator significantly reduces the circulating platelets available for proper coagulation to occur in the patient (5).

Platelet consumption in membrane and hollow fiber oxygenators in the cardiopulmonary bypass circuit is a well-known phenomenon and is widely documented in the literature (2, 6, 7). Despite the combined usage of heparin, protamine, and aprotinin during extracorporeal membrane oxygenation (ECMO) or cardiopulmonary bypass, hemorrhage is still a major post-operative and post-ECMO complication (8, 9, 10). This increased bleeding is attributable to the increased platelet consumption as stated above and to the impaired function once they are exposed to the large artificial surface of the bypass circuit (2, 6, 11). Platelets become activated when exposed to this foreign surface. Clots are then formed, and this chain of events

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results in microembolic complications that may involve hepatic, renal, and pulmonary damage in addition to increased blood loss (6, 12). Over time, platelet aggregation in the oxygenator, if excessive, will cause alterations in the blood flow through the oxygenator and may impair gas exchange in the device (2). When specifically looking at cardiopulmonary bypass, the rapid reduction in circulating platelet exceeds the decrease that can be explained by hemodilution alone (5). Along with foreign surface activation of platelets, the combination of loss of platelet number and function may be responsible for the large proportion of blood loss post bypass (13). When looking at platelet consumption in ECMO circuits specifically, platelet dysfunction can be attributed to, foreign surface exposure, oxidative stress, from exposure to high oxygen tensions and platelet exhaustion (14). Despite nationwide use of heparin, protamine, and amicar, patients continue to experience bleeding complications (14). Most ECMO centers have policies that consist of maintaining platelet counts while on pump at 100,000 or greater and an ACT of 190–210, to prevent bleeding complications (14). A study done in Canada by Cheung et al. specifically investigated platelet function and bleeding complications occurring in infants on ECMO while following the above protocols. One of their study patients was a 37 week, 2200 gm birth weight, with a diagnosis of congenital diaphragmatic hernia, who had a interventricular (brain) hemorrhage 12 h post-ECMO initiation despite the lowest platelet count of 93,000 and an ACT of 200. This example alone demonstrates not only the theory of platelet loss during ECMO but also the occurrence of platelet dysfunction. This patient had an adequate platelet number, but the function of these circulating platelets may have been impaired (14).

Until 1987 nitric oxide (NO) was viewed mainly as a toxic gas found in smog, cigarette smoke, and car exhaust. In late 1987, NO was identified as an endothelium-derived relaxing factor (EDRF) (15). Nitric oxide is now known not only as a vasodilator but also a compound gas that exhibits major roles in smooth muscle relaxation, platelet inhibition, free radical scavenging, neurotransmission, tumor cell lysis, hormone release, and a role in immune function (16). NO is a colorless gas, which is moderately soluble in water. The NO molecule is small, has an unpaired electron, and is a highly reactive free radical. NO is lipid soluble, allowing it to rapidly cross cell membranes. In vivo, NO has a half-life of 0.1–5 sec. NO was first used to treat pulmonary hypertension in 1990 and adult respiratory distress syndrome (ARDS) in 1993 (15, 17). It is still widely used as a treatment modality for pulmonary hypertension, ARDS, cardiac and lung transplantation, congenital diaphragmatic hernia, persistent pulmonary hypertension of the newborn (PPHN), high-altitude pulmonary edema, and chronic lung disease (15). NO is formed endogenously in the endothelial cell with L. arginine as the

precursor (16). NO acts on many proteins, and it is best known for its activation of soluble guanylate cyclase to produce guanosine 3,5, cyclic monophosphate (cGMP). It is the cGMP that mediates the relaxation of smooth muscle and inhibition of platelet aggregation (15). All nitrovasodilators, including nitroglycerin and nitroprusside, cause vasodilation through the production of NO (15).

Humans expire about six parts per billion of nitric oxide at rest and more with exercise (15, 19, 20) The exact origin of this NO is unknown, but evidence has shown that exhaled NO originates in the lower airways (21). An abnormally high or low production of nitric oxide in the body may demonstrate adverse consequences for the patient. A decrease in endogenous NO production may result in systemic or pulmonary hypertension, atherosclerosis, vasospasm, or angina (22). An abnormally high level of endogenous NO is associated with rheumatoid arthritis, hypotension, and increased vasodilation (22). Excretion of NO occurs in both the lungs and the kidneys, with the majority of the loss occurring in the kidneys. Nitric oxide is metabolized by the uptake into the red blood cells where it is converted into nitrate and methemoglobin. Nitrate then enters the plasma and is excreted by the kidneys (23).

Endogenous NO has vasodilator effects on both the systemic and pulmonary circulations; in contrast, inhaled NO only dilates the pulmonary smooth muscle. Any NO that diffuses into the blood path is rapidly inactivated by hemoglobin to form methemoglobin (23). This conversion in the body to methemoglobin results in the lack of systemic dilatory effects to the patient (24, 25). However, excessive methemoglobin formation in the blood may seriously diminish the oxygen-carrying capacity of the blood (26). The safe maximum level of methemoglobin in the blood is only 3% of the total hemoglobin (26). In a study completed by Rimar and Gillis (21), demonstrated the limiting of vasodilation in the pulmonary vasculature by the inactivation of NO by hemoglobin.

Recent research has demonstrated nitric oxide protective qualities to platelets. Researchers have now looked at two methods in which to add the NO to the ECMO and cardiopulmonary bypass (CPB) circuit. These two methods include blending the NO with the sweep gas to the oxygenator circuits (6, 7, 11) and coating the circuit with nitric oxide releasing polymers (16).

Nitric oxide has been well documented as a potent inhibitor of platelet activation induced by foreign surfaces, and because NO is a gas, it can be easily administered to the sweep gas of the ECMO / CPB circuit (25). As compared with other attempts to protect platelets while on bypass; that is, heparin bonded circuits or the use of aprotinin, the use of nitric oxide might be advantageous. First, NO can be easily administered to the gas compartment of the oxygenator. Second, the NO action is restricted at the site of the blood gas interface because of its rapid half life

(27) and bonding ability with hemoglobin. Last, administration of NO can be easily initiated or discontinued when desired. Of the nine studies with NO blended with the sweep gas, the NO concentrations ranged between 15–200 ppm, and the time on bypass varied between 4–24 hours. All of these studies showed a statistically significantly higher circulating platelet count in the NO circuits than in the control circuits (27). Noted in a study completed by Keh (26), in which 20 ppm of NO was used, the protection of platelets increased with the time on bypass. In their study, after 270 minutes on bypass, platelets in the NO circuits were still at 96% of the pre-bypass counts as compared to 82% in the control circuits. It was also noted, in another study by Keh et al. (7), that their nitric oxide circuits not only inhibited platelet adhesion and aggregation to the foreign surface, but also preserved platelet function. In a study done by Sly et al. (12), the oxygenator itself was examined post-bypass for the presence of platelet adhesion. The membrane was X-rayed utilizing a medical scanning device to look for platelet adhesion after infusing 500 ppm for the duration of the trial. Their results demonstrated that 500 ppm showed the maximum platelet adherence to the oxygenator to only 4% of the total platelet count. This finding was also reflected in higher circulating platelet counts (12).

Mellgren et al. (11) used the in vitro study results demonstrating nitric oxide effectiveness in protecting platelets in number and function, and applied these data to human clinical trials. The study performed by Mellgren et al. (11) included 20 coronary bypass graft (CABG) or valve replacement patients, 10 patients received 40 ppm of NO to the oxygenator, and 10 were control patients. The mean bypass time was 73 min, and all patients included in this study were considered a low risk surgical patient with an ejection fraction no less than 50%. This study did show a slightly lower degree of platelet consumption and a higher post-bypass platelet count in the NO group as compared to the control, but these differences did not reach statistical significance. Despite the lack of significance, Mellgren (11) suggests that adding NO to the sweep gas in the CPB circuit will protect platelets while on bypass. In the second clinical trial by Lowson et al. (27), demonstrated similar results as the Mellgren study. Lowson et al. (27) used a patient group of 47 low-risk CABG patients. The NO group received 100 ppm during the duration of the case. This study also discovered a higher platelet count but it did not reach statistical significance.

CONCLUSION

The studies available regarding nitric oxide have thoroughly covered the benefits to platelets in various types of extracorporeal situations. Although the clinical trials with CABG patients did not reach statistical significance, they

have provided direction for further research. Further studies may look at time on bypass, and using different doses of NO to the oxygenator. The protective effects of nitric oxide on platelets did increase with time on bypass. This benefit may prove to be essential in the management of the ECMO patient, whose bypass times are markedly longer in duration than the CABG patient.

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