Introduction

The ductus arteriosus is a large fetal vessel connecting the pulmonary artery with the aorta and allowing right ventricular blood to bypass the unexpanded lungs. At birth, with the start of lung ventilation and the attendant rise in blood oxygen tension, the ductus closes and the cardiovascular system acquires its final arrangement. However, in the prematurely born infant, this shunt may remain patent (patent ductus arteriosus – PDA) with adverse consequences on hemodynamic homeostasis. Conversely, there are cardiac malformations in which patency of the duct is required to maintain the pulmonary or systemic circulation prior to corrective surgery. Based on the notion that patency is an active process sustained primarily by prostaglandin (PG) E₂, PDA is currently managed with synthesis inhibitors, indomethacin or ibuprofen, while any necessary persistence of the duct after birth is achieved with the infusion of PGE₁. However, the former procedure presents a relatively high incidence of failures for the likely combination of the 2 events: the relaxing influence of the agents compensating for the loss of PGE₂ and the immaturity of the oxygen-triggered contractile mechanism. On the other hand, PGE₁ treatment loses some of its efficacy with time and may also be complicated by troublesome side effects. This article presents possible new approaches to therapy still based on the manipulation of the relaxing mechanism(s) responsible for duct patency. At the same time, however, the idea is put forward that the management of these sick infants may find its definitive solution only with tools being designed on the operation of the oxygen-sensing/effector system.

Therapeutic manipulation of the ductus arteriosus: current options and future prospects

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KEY WORDS
ductus arteriosus, endothelin, fetal and neonatal physiology, oxygen sensing, prostaglandin

ABSTRACT

The ductus arteriosus is a large fetal vessel connecting the pulmonary artery with the aorta and allowing right ventricular blood to bypass the unexpanded lungs. At birth, coincidentally with the natural elevation in blood oxygen tension, the ductus closes and the cardiovascular system acquires its final arrangement. However, in the prematurely born infant, this shunt may remain patent (patent ductus arteriosus – PDA) with adverse consequences on hemodynamic homeostasis. Conversely, there are cardiac malformations in which patency of the duct is required to maintain the pulmonary or systemic circulation prior to corrective surgery. Based on the notion that patency is an active process sustained primarily by prostaglandin (PG) E₂, PDA is currently managed with synthesis inhibitors, indomethacin or ibuprofen, while any necessary persistence of the duct after birth is achieved with the infusion of PGE₁. However, the former procedure presents a relatively high incidence of failures for the likely combination of the 2 events: the relaxing influence of the agents compensating for the loss of PGE₂ and the immaturity of the oxygen-triggered contractile mechanism. On the other hand, PGE₁ treatment loses some of its efficacy with time and may also be complicated by troublesome side effects. This article presents possible new approaches to therapy still based on the manipulation of the relaxing mechanism(s) responsible for duct patency. At the same time, however, the idea is put forward that the management of these sick infants may find its definitive solution only with tools being designed on the operation of the oxygen-sensing/effector system.
newborn. In brief, it has afforded a tool, palliative in character but life-saving in finality, to manage certain cardiac malformations with a duct-dependent pulmonary or systemic circulation prior to corrective surgery. Furthermore, the possibility was introduced to promote the closure of a persistent duct (PDA) in the prematurely born infant with an inhibitor of prostaglandin synthesis, specifically a cyclooxygenase (COX) inhibitor such as indomethacin or ibuprofen. Since then, however, this therapeutic approach has remained essentially unchanged despite the many advances made, within and without the ductus, in our knowledge of the vasoregulatory mechanisms. Hence, it has become increasingly out of pace with the data accrued and, by extension, with a need of reassessment.

Prenatal patency Two facts have drastically changed our view of this subject and they relate to the identification of additional vasoactive agents within the ductus and the better knowledge of the functional organization of the PGE, system. For several years, in fact, PGE, was regarded as the only relaxant responsible for the patency of the vessel. Then, in a sequence of advances, perhaps not complete as yet, evidence has been obtained on the contribution of nitric oxide (NO), carbon monoxide (CO), and lately hydrogen sulfide (H,S). Furthermore, the potential to generate the hitherto uncharacterized endothelium-derived hyperpolarizing factor (EDHF) under certain conditions has also been ascertained. These agents are closely intertwined in their operation and may complement each other thanks to differences in their physicochemical properties (PGE, and CO: stable; NO, H,S, EDHF: labile) and a likely uneven developmental profile. For example, NO is more active relatively to PGE, in the preterm, while this reciprocal relationship acquires an opposite sign at term. Furthermore, according to our data, suppression of COX, either by gene deletion or pharmacological means, is followed by NO upregulation. Exemplary in this context is also the arrangement in the guinea pig where, for a peculiar evolutionary departure, the ductus is missing the PGE, -based relaxing mechanism but still remains patent antenatally as in any other species. The inevitable conclusion in such case is that PGE, function is taken over by 1, or more than 1, of the allied agents. In sum, several endogenous factors may act in concert with PGE, to maintain ductus patency, and this notion has important consequences for the clinic. Compounding this problem is also the recent report of a possible role of the isoprostanes, that is of vasoactive compounds being generated nonenzymatically from arachidonic acid. If validated, the finding would introduce a further degree of complexity because, by their nature, these prostanooids are not amenable to inhibition from drugs, such as indomethacin and ibuprofen, interfering with the COX function.

The role of PGE, has also become increasingly complex with the realization that its action within the ductus is not limited to muscle relaxation. In brief, it has been shown that PGE, also contributes to closure of the duct, antenatally by promoting the formation of intimal cushions, which are critical for the sealing of the lumen, and postnatally by being involved in remodeling of the vessel wall, that is, in the process leading to structural closure and, ultimately, to the demise of the shunt. In essence, one is dealing with an agent exerting opposite effects, definitely different in their time course but still capable to introduce some degree of unpredictability in the response to a synthesis inhibitor. In addition, over the years, the synthetic system for PGE, has revealed its full complexity, first with the identification of 2 isomers, COX-1 and COX-2, in what was originally perceived as a single entity, and second, with the characterization of terminal enzymes in the synthetic cascade (i.e., the PGE synthases). The ductus is endowed with a complete, COX and PGE synthase, system, hence making it unrewarding to attempt to replace currently used nonselective inhibitors with the newly developed COX-2 inhibitors. Conversely, as discussed below, a promising lead may be found in the PGE synthase complex when considering that one of its constituents, microsomal PGE synthase-1 (mPGE1), is the prime source for PGE, in the ductus and that mPGE1 suppression, unlike suppression of either COX or PGE, is not followed by NO upregulation.

Postnatal closure The functional closure of the ductus rests on 2 complementary events, the contractile effect of oxygen on the muscle cells and the lesser influence of relaxant PGE, that follows the abrupt cessation of the placental function (i.e., the major source for blood-borne compound) and the waning expression of local PGE, receptors including the critical EP, subtype. A considerable effort has been devoted over the years to identify the oxygen sensor and elucidate the mechanism by which its activation is translated into sustained contraction. Peculiar in many ways, the sensor recalls in its operation a conventional receptor, and this feature could provide means for a targeted manipulation. Specifically, it shows a selective distribution, with only few sites sharing such property with the ductus; a developmental profile which is independent from the maturation of the contractile function; desensitization upon repeated or extended exposure to the agent; and an accelerated expression upon treatment with certain compounds, particularly retinoic acid.

Two schemes have been proposed for the oxygen effector mechanism and they are not mutually exclusive. In fact, they may in some way complement each other. According to our scheme, oxygen acts on a special cytochrome, P450 (CYP450), belonging to the glucocorticoid inducible 3A subfamily and...
located for better accessibility in the plasma membrane of the muscle cells. In the mouse, this heme-protein has been identified with CYP3A13 which, among the several isofoms of the murine subfamily, bears the greatest homology with the human CYP3A.20 Once activated, the CYP450 sensor would function as a catalytic element in a monoxygenase reaction from which a product is generated serving as a link with the constrictor endothelin-1 (ET-1). Therefore, ET-1 is regarded as the ultimate effector for oxygen. The identity of the coupling agent between sensor and effector remains to be ascertained. However, our work to date identifies this putative messenger with an arachidonic acid metabolite originating from a concerted interplay between the CYP450 epoxygenase and 12(S)-lipoxygenase pathways.20

Alternatively, the oxygen sensing function has been ascribed to a mitochondrial redox mechanism, located in the muscle cells and producing a signal in the form of reactive oxygen species. This redox messenger, expectedly hydrogen peroxide, would elicit a contraction by inhibiting a set of voltage-gated potassium channels (i.e., Kᵥ1.5 and Kᵥ2.1) and causing an activation of voltage-dependent L-type and T-type calcium channels through the ensuing depolarization.28 A recent development in this scheme has been the identification of a key factor (dynamin-related protein 1 [Drp1]) conditioning the generation of the redox signal.31

In this context, it is significant that interference with either of the proposed sequences results in subsidence of the oxygen response.28,31 Hence, CYP450 inhibitors of diverse structure as well as the suppression of the ET-1 function are effective in this respect.28 Congruent with this notion is also a recent report showing, on one hand, that cimetidine represents a risk factor for PDA in the prematurely born infant and, on the other, that the same drug interferes with the ducus constriction to oxygen by inhibiting a CYP450-based mechanism.32 By the same token, Drp1 inhibition is effective in curtailing selectively the oxygen response.31 The 2 proposals for oxygen sensing present other common features.28 Clinically relevant is the fact that both mechanisms are susceptible to facilitation by glucocorticoids. Indeed, glucocorticoids contribute to the maturation of oxygen sensing8,33 and, accordingly, their use in the perinatal period reduces the incidence of PDA.34 In sum, the evidence accrued to date points to the likely existence of 2 mechanisms for the ducus response to oxygen, conceivably overlapping at some point and, yet, being amenable to distinct manipulations. Significantly, either of these putative sensors can be transfected into vascular muscle cells, which, although intrinsically unresponsive, become susceptible to oxygen.35,36 The latter possibility is not only conceptually important but may also open the way to targeted manipulations of the sensor with the purpose of developing new therapeutic tools.

Whatever the target for oxygen, functional closure evolves naturally into a permanent occlusion of the duct. Several events contribute to vessel remodeling through this transition,23 but it is beyond the scope of this article to delve into details. Here, it suffices to say that PGE₂ is definitely involved in this process28 and that ET-1 is likely to play a role too in view of its mode of action elsewhere.

**Clinical implications** A clear message from the previous sections is that mechanisms governing the ducus arteriosus, whether antenatally or postnatally, are redundant and comprise complementary agents being controlled by independent pathways. Hence, the vessel presents a dynamic system with the capability of adapting to, and eventually overcoming, diverse solicitations. A definite advantage, for example, in protecting the fetus from possible insults. However, this versatility may also become a problem for therapy, particularly if the tools in use rest on the assumption (now proven fallacious) that PGE₂ exerts its control alone and with only 1 mode of action, as it is in our case. Nevertheless, on the bright side, the same situation affords new opportunities and, in fact, a potential for therapy that is still largely unfulfilled. This ample perspective will guide our analysis of the present protocols for ducus manipulation in infants with prematurity-linked PDA and duct-dependent congenital cardiac malformations.

**Persistent ducus arteriosus of the premature** Current therapy, based on COX inhibitors, is far from optimal owing to a relatively high incidence of failures, particularly among the youngest infants of the group, and the risk of troublesome side effects.27 This is a heavy burden, indeed, considering the magnitude of the problem of prematurity worldwide.31 The reason for limited success is likely multifold. Not only is the oxygen-sensing mechanism inadequate in providing a contractile drive, but also the removal of the PGE₂-based relaxation occurs in parallel with events that counter the closing process, such as the upregulation of NO and interference with wall remodeling. In fact, these counteractive influences are strong enough to make antenatal exposure to COX inhibitors a risk for PDA.29,40 The problem is further complicated by events, proper of the physiology and pathophysiology of the newly born, that may variably condition the effectiveness of inhibitors. The normal rise in blood oxygen tension at birth, although unable to cause the closure of the shunt, may promote the formation of certain vasorelaxants, specifically those with oxygen-dependent synthesis (i.e., PGE₂, NO).41,42 Likewise, the mechanical solicitation, imposed on the vessel wall at birth by the abrupt reversal in the direction of blood flow across the duct as well as by any turbulence subsequent to the narrowing of the lumen, whether spontaneous or drug-induced, is a possible cause for NO upregulation.43,44 Any
intervening infection and certain drugs commonly used with the sick infant are also factors to be considered in this context. Pyrogens can up-regulate, in fact, the whole cohort of vasorelaxants formed in the ductus. Cimetidine and possibly other drugs may oppose the closure process with their dilator action. Then, it should not be surprising that COX inhibitors have often a transient effect or no effect at all. Having recognized the problem, so far 2 main approaches have been suggested as a solution, mostly in an experimental setting. One way has been to target discrete components of the PGE₂ system with the purpose of obtaining a more selective response with a lower risk of adverse consequences, particularly for the fragile hemodynamic status of the premature. Hence, this selectivity has been sought by testing either an antagonist of the PGE₂ receptor subtype EP₄ or an inhibitor of the terminal mPGE₁ enzyme in the synthetic cascade. As expected, both treatments constrict the ductus with the distinct advantage over COX inhibition of leaving PGI₂ (prostacyclin) function unaffected. In truth, there has been some inconsistency in the response to the mPGE₁ inhibitor, but its cause is likely connected to the particular compound used. On the other hand, the absence with mPGE₁ inhibition of any rebound upregulation of NO is quite advantageous. On the downside, however, both agents still have opposing effects on ductus closure – i.e., subsidence of muscle relaxation along with inhibition of tissue remodeling – because this dual feature is intrinsic to PGE₂ action via the EP₄ receptor. Nevertheless, the therapeutic potential of these agents warrants further investigation. An alternative approach for better efficacy has been to combine the COX inhibitor, specifically indomethacin, with an inhibitor of NO synthase, hoping to reproduce at the bedside a model of treatment solidly supported by conceptual considerations and experimental findings. However, a clinical trial with this protocol, while showing an immediate response by the ductus, had to be stopped due to troublesome side effects. In addition, the vessel tended to reopen despite its firm constriction at the start of treatment, possibly for the overriding influence of alternative relaxant agents. Lastly, one should mention in this context the clinical use of paracetamol in the place of indomethacin (or ibuprofen). Compared with proper COX inhibitors, paracetamol acts upon the peroxidase end of the COX complex and is thought to be safer. However, the experience to date, which includes some conflicting results on the actual efficacy of the drug, is limited and does not allow to draw firm conclusions. In sum, several strategies, already tried at the bedside or still experimental, have been proposed for treatment of PDA in place of indomethacin (or ibuprofen). However, their common weakness is an inability to suppress all relaxing mechanisms and the expected interference with the structural phase of the closing process.

From the foregoing, it becomes clear that a true advance may only come from the development of mechanism-based tools capable of duplicating in the premature the forceful contraction exerted by oxygen at term. An attempt in this direction has been made by demonstrating, experimentally, the great efficacy of a thromboxane A₂ mimic in inducing constriction of the ductus. However, translation of this finding to the clinic is hardly feasible in view of the generalized action of the compound and its prothrombotic properties. Conversely, one should start considering tools for ductus closure that reproduce the normal operation of the oxygen-sensing mechanism, taking advantage in so doing of the growing knowledge on the subject. After all, the “receptor-like” arrangement being assigned to the oxygen sensor, lends itself to a targeted intervention. At this point in time, any elaboration on the actual direction to be taken is by necessity highly speculative. However, a useful lead might be found in the characterization of the putative messenger linking the CYP450-based oxygen sensor with the ET-1 effector. Equally rewarding might be the exploitation for the same purpose of the critical role being assigned to Drp1 and its related kinases in the oxygen response. Furthermore, in the same vein, a more incisive investigation of the mechanism(s) by which certain agents (i.e., retinoic acid, glucocorticoids) promote the maturation of the oxygen sensor could prove useful. Surely, this seems the proper approach for the future, notwithstanding the challenges in sight.

**Ductus-dependent cardiac malformations** Exemplary in this clinical context are right-heart obstructive malformations and interrupted aortic arch as they require a patent ductus to sustain, respectively, pulmonary and systemic circulation. PGE₁ was first introduced as an effective ductus dilator to stabilize temporarily the condition of these sick infants prior to corrective surgery, thus converting this intervention from an emergency into an elective affair. With time, however, a protracted administration of PGE, has become desirable in diverse instances, and this has brought into light some negative features. Specifically, a decline in the efficacy of the compound and the greater impact of side effects, of which apneic spells are the most prominent. This change in PGE, activity should not come as a surprise. After all, the steady condition of the patient and, in particular, a normal or quasi-normal blood oxygenation sets into motion the mechanisms that normally initiate closure of the duct. Hence, the PGE, being administered must operate against this opposing force. Furthermore, PGE, itself, besides relaxing the vessel, promotes the remodeling process that is ultimately responsible for occlusion of the shunt. To overcome this problem, it has been thought to magnify the action of endogenous PGE, and allied relaxants by suppressing the enzymatic breakdown of their second messenger. Hence, inhibitors of
phosphodiesterase 3 (PDE3; amrinone, milrinone)\(^56,57\) and 5 (PDE5, sildenafil)\(^58,59\) have been tested experimentally with the purpose of increasing the lifespan of cyclic adenosine monophosphate (for PGE,) and cyclic guanosine monophosphate (for NO and possibly CO). As expected, either intervention dilates the duct, with PDE3 inhibition being more effective than PDE5 inhibition, particularly in reversing the closure of the vessel after birth.\(^56,57\) Nevertheless, before considering any transfer of such knowledge to the clinic, one should note that the PDE3 inhibitor may not be that useful over an extended period of time. In fact, however magnified, the PGE, action could still elicit opposing responses via a common cAMP messenger, and a suggested possibility of separating these 2 actions for clinical purposes\(^57,60\) would require verification with a chronic experimentation in vivo. A different situation is envisaged with the PDE5 inhibitor, because its action, involving NO and possibly CO, may truly complement that of PGE,. Nevertheless, this synergy will be limited to the relaxant component of the response, while structural changes induced by PGE, may still proceed unabated.

A reasonable conclusion from the foregoing is that suppression of the oxygen-induced constrictor mechanism, either by itself or in some combination with PGE, treatment, might be the best option for ensuring long-term patency of the duct. One possibility would be to counter the action of ET-1 with an appropriate inhibitor, hence bringing to bear experimental data in favor of an effector role for this agent.\(^28\) Particularly appealing in this case would be the predicted feasibility of interfering with both functional and structural closure. Alternatively, as it has been proposed for the management of PDA, one should take advantage of the better knowledge of the oxygen sensing mechanism and, accordingly, consider novel ways for its curtailment. In principle, several leads are worth pursuing for a targeted inhibition. For example, the putative CYP450 sensor, belonging to the 3A subfamily, may be blocked, selectively and with remarkable potency, by the protease inhibitor, ritonavir.\(^31,62\) Significantly, the target site for the drug has been characterized\(^63\) and could provide a useful clue for any further development. Equally rewarding might be an approach based on the critical role being assigned to Drp1 in the oxygen response.\(^31\) In this context, a recent work point to the possible design of a selective inhibitor for the sensor-linked potassium channel, Kv 1.5, is worth noting.\(^64\) In other words, several options are at hand to meet this particular challenge.

**Conclusion** The concept of ductus patency being an active process sustained by PGE, has been a turning point for neonatal care. Immediate applications have emerged from this with the introduction of PGE, as a life-saving tool to maintain ductus patency in certain cardiac malformations of the newly born and, conversely, with the use of COX inhibitors to close the duct in the preterm infant. Both procedures, however, present drawbacks, and possible improvements are presented here that take advantage of the growing knowledge of the relaxant mechanism. Still, a true advancement is envisaged with the development of novel tools being designed on the operation of the oxygen-based contractile mechanism. This is indeed a formidable challenge, where success would reaffirm the eminently translational character of research in this area.

**Note** A paper has recently been published that reaffirms the prime role of EP, mediated PGE, action in remodeling of the duct.\(^65\) This PGE, /EP, signaling is known to be located in the muscle.\(^66\)

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ARTYKUŁ POGLĄDOWY

Interwencje terapeutyczne w zakresie przewodu tętniczego – dostępne opcje i widoki na przyszłość

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STRESZCZENIE

Przewód tętniczy to duże naczynie płodowe łączące tętnicę płucną z aortą, pozwalające krwi tłoczonej przez prąd komorę ominąć nierozprężone płuca. Po porodzie, wskutek rozpoczęcia wentylacji płuc i związanego z tym wzrostu ciśnienia parcjalnego tlenu we krwi, przewód tętniczy zamyka się, a układ krążenia osiąga swój ostateczny kształt. Jednakże u niemowląt przedwcześnie urodzonych przewód ten może pozostać drożny (patent ductus arteriosus – PDA), co ma niekorzystne następstwa dla homeostazy układu krążenia. Z drugiej strony istnieją drożne wady serca, w których drożność przewodu tętniczego jest konieczna do utrzymania krążenia płucnego lub systemowego do czasu korekcji chirurgicznej. W związku z tym, że utrzymanie drożności przewodu tętniczego jest czynnym procesem, uruchamianym głównie przez prostaglandynę (PG) E₂, w leczeniu PDA stosuje się obecnie inhibitory syntez PG – indometacynę lub ibuprofen, natomiast niezbędną drożność po porodzie utrzymuje się za pomocą wlewu PGE₁. Niestety ta pierwsza metoda obarczona jest dość dużym odsetkiem niepowodzeń, za co może odpowiadać skojarzenie 2 czynników: relaksacyjnego wpływu substancji kompensujących niedobór PGE₂ oraz niedojrzałości mechanizmu obkurczającego zależnego od tlenu. Z kolei leczenie PGE₁ z czasem staje się coraz mniej skuteczne i może być powikłane uciążliwymi objawami niepożądannymi. W artykule omówiono nowe możliwości leczenia, w dalszym ciągu opartego na ingerencji w mechanizmy relaksacji odpowiedzialnej za drożność przewodu tętniczego. Równocześnie jednak przedstawiono hipotezę, że definiujące rozwiązanie problemu postępowania u tych chorych niemowląt umożliwi dopiero wykorzystanie narzędzi pozwalających na ingerencję w sensoryczne i efektorowe składowe mechanizmu reakcji na tlen.

SŁOWA KLUCZOWE

endotelina, fizjologia płodu i noworodka, prostaglandyna, przewód tętniczy, receptory tlenu