New Inotropic Pharmacologic Strategies Targeting the Failing Myocardium in the Newborn and Infant

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Abstract: Pharmacologic support of the failing neonatal heart to maintain cardiac output, which is vital for sufficient end organ perfusion, is a challenging task for the pediatric intensivist, especially since strategies which have been proven to be effective in adults cannot necessarily be extrapolated to neonates. The unique biochemical properties and structure of the neonatal heart, including the increased non-contractile tissue mass, a lower responsiveness to beta adrenergic agents and the heart rate dependent cardiac output with a limited ability to increase stroke volume, favor some of the new inotropes of the Ca+ sensitizer family. Focusing on the after load reduction, inodilators as phosphodiesterase inhibitors and human brain natriuretic peptide offer treatment options for the neonatal myocardium. Additionally, thyroxine and steroids have been investigated in neonates with low cardiac output after surgery for congenital heart disease. Gene therapy, in particular cardiac-selective gene transfer, might offer perspectives for future support for the neonatal heart. This text reviews some of the most recent pharmacologic strategies targeting the failing myocardium in the critically ill newborn and infant.

INTRODUCTION

In sick neonates and infants, the unique biochemical properties and structure of the heart including the increased non-contractile tissue mass, low compliance of the ventricle with resulting increased sensitivity to preload, a lower responsiveness to beta adrenergic agents plus the heart rate dependent cardiac output with limited ability to increase stroke volume, make it difficult to extrapolate data on inotropic support for the failing myocardium from the adult to the newborn heart.

In contrast to adults, immature neonatal cardiomyocytes lack transverse tubules and are smaller in size. The sarcoplasmatic structures are underdeveloped and L-type calcium channels and sarcoplasmatic calcium release channels are physically separated. Consequently, calcium-induced calcium release is markedly diminished. Immature cardiomyocytes are therefore much more reliant on trans-membrane calcium flux for contraction and relaxation. The sodium-calcium exchange is the major transport pathway for provision and removal of calcium from the contractile proteins in the newborn heart.

In infants with congenital heart disease (CHD), low cardiac output syndrome (LCOS), defined as a cardiac index of ≤ 2.0 L/min/m^2 despite adequate filling pressures defined as a left atrial pressure exceeding 8 mm Hg, is the most frequently occurring peri-operative complication. The incidence of LOCS in neonates undergoing surgery for CHD is repeatedly reported to be as high as 25% [1].

In these infants, β-agonists are still the most frequently used compounds to restore adequate cardiac output. The drawbacks of this treatment are the down regulation of β-receptors, increased oxygen consumption of the myocardium, combined with an increased peripheral resistance (increased afterload) and impaired diastolic function [2,3]. The latter is the result of the common principle of β-agonists, modulators of the sodium/potassium pump and to some extent phosphodiesterase inhibitors (PDE), to increase intracellular Ca^+ concentrations. While increasing contractility in systole, this increase in Ca^+ severely impairs relaxation in diastole, potentially inducing arrhythmia and negatively influencing cell survival. Thus, these agents have shown little success in reducing mortality in randomized clinical trials in adults [4].

This text reviews the biochemical and pharmacologic characteristics as well as the available evidence of the most promising agents emerging to treat the neonate and infant in cardiac failure.

PHOSPHODIESTERASE INHIBITORS

Amrinone, enoximone and their derivatives as well as milrinone, are bipyridine compounds (Fig. **1**) that selectively inhibit cyclic nucleotide phosphodiesterase (PDE) III and lead to an increase in intracellular cyclic AMP independent of β-adrenegic receptor stimulation. Of the several PDEs detected in the myocardium, including a Ca2/calmodulin–activated PDE, a cyclic guanosine 3,5 mono-phosphate (cGMP)–stimulated PDE, and a low-Km, cGMP–inhibited PDE, the latter is most strongly inhibited by milrinone. The main mechanism of action of milrinone is, as in most of the here discussed agents, multiple. The increase in myocardial contraction is result of an altered trans-sacrolemal Ca^+ flux [5], the decrease in peripheral vascular resistance is a result of an increased uptake of Ca in the sarcoplasmatic reticulum and therefore a decrease in free intracellular Ca^+ in the smooth muscle cells of the vasculature [6] and finally, some improvement in lusitropy

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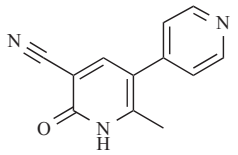

Fig. (1). Milrinone.

is considered to be a result of an enhanced dissociation of actin and myosion during diastole [7]. The vasodilatory effects of milrinone are magnified in the presence of and β - adrenergic agents such as phenylephrine, norepinephrine, or dopamine because of the combined activation of adenylate cyclase and inhibition of phosphodiesterase III [8,9],. In the rare scenario of milrinone-induced, norepinephrine resistant hypotension, the administration of vasopressin may be necessary.

Reversal of thromboxane A_2 induced vasospasm by milrinone has been observed in ex-vivo models. In one study, performed in adult cardiac surgery patients, decreased levels of hepatic and venous endotoxin and interleukin 6 compared to placebo were observed [10], although the suggested anti-inflammatory effect of milrinone, other than improving splanchnic perfusion, remains unclear. Other studies report an anti-inflammatory action of amrinone, while this effect could not be proven for milrinone [11]. In contrast, a recent study has described an up-regulation of NFκB transcription by the inhibition of PDE 3 as well as 1 and 4 while inhibition of PDE 2, 5 and 6 down-regulated NFκB transcription *in vitro* [12].

Amrinone, enoximone and milrinone have been used successfully in adults and children; however, data from animal studies have raised some concern regarding the efficacy of PDE inhibitors in neonates. A number of studies in neonatal cat, rat, avian and rabbit cardiomyocytes failed to show any positive inotropic effects of amrinone [13], and studies in neonatal piglets even demonstrated a decrease in cardiac performance [14]. These findings might reflect the relatively low intracellular PDE concentration in the neonatal heart. Milrinone, for some reason, did not show such negative effects compared to amrinone in neonatal piglets [15]; however, the positive inotropic effect of milrinone is also clearly age- dependent [16].

The half-life of milrinone is longer than that of catecholamines, therefore pointing towards the need for bolus administration before continuous infusion, while pharmacokinetics of the first 12h is mainly determined by redistribution (higher volume in infants and children compared to adults) and is approximately 30 min after 30 min of infusion and increases to 140 min when saturation has occurred [17].

In contrast to many other medications used in neonates to counteract LCOS, milrinone was investigated in a randomized clinical trial in this population [18]. In this trial, 238 infants and children were randomized into three groups, one placebo, one starting with a milrinone bolus of 25 μg/kg over 60 min, followed by 0.25μg/kg/min for 35h and one starting with a milrinone bolus of 75 μg/kg over 60 min, followed by 0.75μg/kg/min for 35h. There was a significant reduction in the development of LCOS in the high dose group with a relative risk reduction of 55% compared to placebo. While amrinone causes marked thrombocytopenia in some patients, which has also been reported although less frequently, for milrinone in adults and children [19], such an effect was not observed in this study. The effect of amrinone on platelets is postulated to be a toxic action of a metabolite, N-acetylamrinone on megakaryocytes, however all PDE inhibitors can increase cAMP generated from adenosine triphosphate by adenyl cyclase. Platelet cAMP is an inhibitor of platelet activation at numerous steps and all PDE III inhibitors can inhibit platelet activation at least *in vitro* [20,21].

A case series of 10 neonates post cardiac surgery, in whom monitoring of hemodynamic variables was performed using atrial catheters in the right and left atrium plus pulmonary artery pressure and thermo-dilution measurements, showed that milrinone lowered cardiac filling pressure, systemic and pulmonary vascular resistance and pressures and that the drug increased cardiac index and heart rate without increasing myocardial oxygen consumption in neonates with post-operative LCOS [22]. Another, recently published study investigated 15 infants within the first 16 month of life with post cardiac surgery LOCS. As in the previous studies, this work also confirmed a direct myocardial effect of milrinone as improvement in biventricular myocardial function measured by Doppler-derived, time interval-based index of myocardial performance [23].

CALCIUM SENSITIZERS

The best studied compound in this new family of inodilators, referred to as Ca^+ sensitizers, is levosimendan (Fig. **2**). The action of levosimendan is via multiple pathways. The main and nominal function is the action on troponin C, which is maintained longer in the configuration to trigger and sustain contraction in the presence of Ca^+ ions [24-26]. The latter detail is of uttermost importance since this facilitates the different action of the drug, promoting contraction in systole and relaxation in diastole [27,28]. An additional effect of levosimendan on Ca^+ uptake and release in the sarcoplasmatic reticulum has been discussed [29].

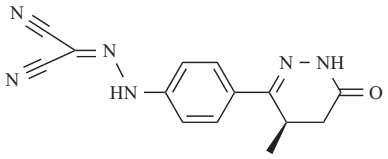

Fig. (2). Levosimendan.

The second important action of levosimendan is the relaxation of the arterial and venous vasculature by an ATP dependent opening of potassium channels in smooth muscle cells. Of special importance for the neonate is this effect on the vessels in the pulmonary circulation and the described decreasing pulmonary vascular resistance in patients treated with levosimendan. Levosimendan also inhibits PDE, however, other than the PDE 3 inhibitors amrinone and milrinone this effect of levosimendan is relatively PDE 4 specific and only high concentrations of Levosimendan

inhibit PDE 3 *in vitro*. In contrast to the PDE 3 inhibitors, levosimendan does not increase intracellular cAMP and Ca^{+} concentrations in pharmacologic doses.

Levosimendan has some positive chronotropic effect, which, since not preventable by β-blockers seems not to be an β-adrenergic mediated function but an effect of PDE inhibition [30]. Levosimendan shortens sinus cycle length and sinus node recovery time, as well as atrioventricular nodal conduction interval and refractory periods [31]. Therefore, a possible pro-arythmogenic effect is, although not reported in adults, conceivable in neonates and this issue should be addressed in future studies. The increase in heart rate is maintained long after the infusion of levosimendan, pointing towards an effect caused by metabolites of the substance. Indeed, 5% of Levosimendan, with a good bio-availability of 98% a half-life of only 1h and elimination via conjugation and excretion via urine and faeces, is metabolized to the active metabolite OR-1896 [32,33]. OR 1896 is formed from an intermediate metabolite OR-1855, which is most likely produced by intestinal bacteria from levosimendan excreted by bile and then reabsorbed [34]. Although the plasma elimination half-life of OR-1896 is reported to be 80h, the long-lasting (1 week) effects of a 24h infusion of levosimendan are questionable in the neonate with an immature hepatic-biliary cycle. In adults, infusion of levosimendan for up to 7 days did not induce tolerance, nor did drug withdrawal result in cardio-vascular rebound effects [34].

Neuro-endocrine functions of levosimendan have been hypothesized, since the plasma concentration of endothelin is decreased while those of natriuretic peptide and noradrenaline are slightly increased [35].

Clinical experience with levosimendan has, up to now, almost exclusively been gained in adults. The two most important therapeutic trials are the Levosimendan Infusion versus Dobutamine in severe low Output heart failure (LIDO) Study [36] and the Randomized Study on Safety and Effectiveness of Levosimendan in patients with Left ventricular failure (RUSSLAN) [37]. In the LIDO trial with a total of 203 patients enrolled, patients treated with levo-simendan showed a more effective increase in CO and decrease in pulmonary capillary wedge pressure (PCWP) compared to those patients receiving dobutamine. Levo-simendan reduced systolic blood pressure and caused more vasodilatation than dobutamine. Additionally, the effects of levosimendan persisted for more than 6h after stopping the drug infusion whereas the effects of dobutamine vanished. In the RUSSLAN study, where 504 patients with pulmonary edema occurring within 5 days after acute myocardial infarction were enrolled, patients were randomized to placebo or four different 6h dosing regimens of levosimendan (A: 10 min bolus 6 μg/kg, followed by 0.2 μg/kg/min; B: 10 min bolus 12 μg/kg, followed by 0.2 μg/kg/min; C: 10 min bolus 24 μg/kg, followed by 0.2 μg/kg/min; D: 10 min bolus 24 μg/kg, followed by 0.4 μg/kg/min). Targeting on safety, the major endpoints in the RUSSLAN study were hypotension and myocardial ischemia, which was not found to be increased in the patients receiving levosimendan except in those in Group D. At 6h and 24h, the exacerbation of LOCS was less pronounced in the levosimendan groups compared to placebo. In both trials, reduction in mortality in those patients treated with levosimendan was significant compared to those receiving placebo at 14 days (RUSSLAN), 31 days (LIDO) and 180 days (LIDO and RUSSLAN).

Studies on levosimendan in infants and neonates are extremely limited. Although randomized clinical trials in this population are completely lacking, some case series and reports have been published. The largest study available is a pharmacokinetic study on 13 children and infants aged between 3 months and 7 years undergoing cardiac catheter-ization prior to cardiac surgery [38]. The children received 12 μg/kg levosimendan as a bolus over 10 min and blood sampling was done within the next 4 h to evaluate distribution (0.24 h mean) and elimination half-life (1.6 h mean, with a trend to longer elimination half-life in children younger than 6 months) which were quite similar to those of adults with congestive heart failure. Hemodynamic effects of the substance, measured invasively during the catheterization and by echocardiography 2h after infusion, were not significantly altered, most probably due to the small dosage applied with respect to the body surfac area. In addition, three case reports on the use of levosimendan in the pediatric population have been published. One reported on a 12- year-old, suffering from myocarditis, with successful weaning from a bi-ventricular assistance device using levosimendan [39]. The two other reports focussed on newborns with CHD and pulmonary hypertension with pulmonary artery pressures exceeding those of the systemic circulation [40,41]. In both patients, levosimendan was given after other pharmaco-logical approaches to decrease pulmonary vascular resistance such as epoprostenol and inhaled NO, failed. In both infants, a clinically significant fall in pulmonary artery pressures was observed combined with an improvement of cardiac output.

HORMONES: NATRIURETIC PEPTIDES

The concept of the heart as an important endocrine organ emerged with the work of de Bolt *et al*. in the early 1980´s [42]. The hormones of cardiac origin first to be described were atrial natriuretic peptide (ANP), followed by brain natriuretic peptide (BNP) and c- type natriuretic peptide (CNP) [43-45]. All the natriuretic peptides have a common powerful action as vasodilators, and promote natriuresis and diuresis. Whereas ANP is mainly synthesized in the atria of the heart, the main source of BNP is the ventricle [46]. The main, but not only, stimulus for the release of the natriuretic peptides from the cardiomyocytes is wall stretch, as induced by volume overload or increased afterload. Other stimuli which increase BNP transcription and release are activation of α_1 adrenergic receptors and exposure to endothelin 1 [47,48]. The latter stimulation is rapid and may compensate for the fact that BNP, in contrast to ANP, is not stored in intra-cellular granula. Indeed, following α_1 adrenergic stimulation with phenylepiphrine and endothelin-1, the transcription of BNP mRNA reaches its maximum within only 1h, pointing towards BNP as an “emergency” hormone against ventricular overload [49].

BNP in particular has received attention as both a marker for cardiac failure and a therapeutic compound. BNP, first isolated from porcine brain, consists of a 32 aminoacid ring

structure with an intra-molecular disulfide bridge in man (Fig. **3**). Synthesised by cardiomyocytes as pro-BNP, cleavage of the inactive NT pro-BNP activates the hormone. There are three different subtypes of natriuretic peptide receptors (NPR) on the cell surface. BNP signals together with ANP through NPR-A which is located on the surface of both endothelial and vascular smooth muscle cells [49]. After binding to its receptor, BNP induces an increase in cGMP and activates Ca^{+} sensitive and ATP dependent K^{+} channels to promote vasodilatation [50]. Interestingly, ANP signaling through cGMP was reported to promote apoptosis *in vivo* and limit fetal cardiac growth *in vivo*, while β-adrenergic stimulation with norepinephrine antagonizes this effect by stimulation of the anti-apoptotic Bcl2 homologue Mcl1 [51]. A similar effect of BNP on apoptosis, although likely, has not yet been described. Another, probably through the natriuretic peptide clearance receptor (NPR-C) mediated effect of BNP, is an enhancement of NO production, further contributing to vasodilatation [52]. The latter might be of special importance in patients with heart failure and down regulated expression of NPR-A [53]. Interestingly, ex-vivo studies on human vasculature have shown a dose dependent shift of BNP action from the venous to the arterial side. Whereas low concentrations of BNP mainly resulted in a venous vasodilatation, higher doses promoted an arterial relaxation and thereby led to afterload reduction [54]. This effect of BNP seems to be an indirect action in which BNP stimulates the endothelial production and release of CNP. CNP, after binding to its receptor which is, at least in newborns, predominately expressed in venules, subsequently signals via an cGMP independent pathway [55].

The plasma half-life of BNP is approximately 20 min, which is significantly longer than that of ANP (3 min) [56]. Although both peptides are eliminated through NPR-C, the difference in plasma half-life suggests other, yet unknown, mechanisms. The density of NRP´s on the cell surface, however, can be down regulated [57]. This might be one explanation of the observed reduced responsiveness to recombinant human BNP in patients with chronic heart failure. Unfortunately, there are no available data on the expression of NRP´s in neonates.

In large clinical trials, recombinant human BNP (nesiritide) was typically compared to treatment with inotropes, mostly dobutamine, While nesiritide was able to reduce left ventricular filling pressure and symptoms of dyspnea compared to placebo, the comparison with dobutamine demonstrated a superior safety profile of nesiritide [58]. However, in patients treated with pharmacologic doses of nesiritide, an increase in serum creatinine, together with an impairment of diuresis was frequently observed [59]. Since this is known to be a negative prognostic maker, even if transient, a recent meta-analysis comparing nesiritide with other, non-inotropic approaches to LOCS, showed a concerning increase in short-term mortality in patients treated with nesiritide [60,61].

In infants with CHD, the compound was investigated in two larger case series and a number of case reports. These reported a (biased) benefit of nesiritide in terms of controlling hypertension and restoring diuresis in children awaiting cardiac transplantation (n=7) [62], children with heart failure (n=30) [63], and patients on a pediatric intensive care unit (n=5) [64]. Unfortunately, in the pediatric population, randomized data neither on the comparison of nesiritide and dobutamine nor on the comparison of nesiritide and non-inotrope treatment are available. Additionally, there are no sufficient data on the course of creatinine in neonates or infants treated with nesiritide to exclude the adverse events observed in adults.

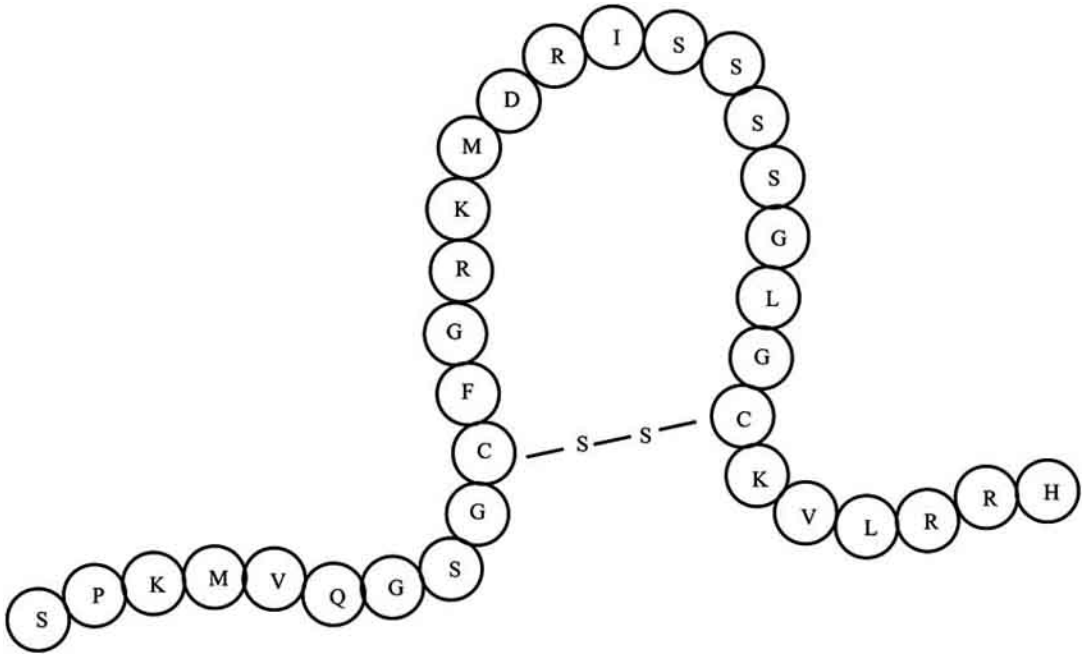

Fig. (3). BNP.

HORMONES: THYOXINE AND STEROIDS

In the late 1980's, many investigations reported that the cardiovascular status of the hypothyroid patient is similar to the cardiac surgery patient in that both exhibit low cardiac output with increased systemic vascular resistance. Additionally, there are many reports on decreased plasma levels of thyroid hormones in adults and children undergoing cardiac surgery with cardio-pulmonary bypass (CPB) and ultrafiltration. Recently, an investigation postulated an association between post CPB systemic inflammatory response syndrome (SIRS) with IL6 elevation and depressed thyroid hormone levels [65]. The heart is highly sensitive to the effects of thyroid hormones and triiodothyronine (T3) (Fig. **4**), the most active thyroid hormone, increases heart rate, cardiac contractility (direct and indirect effects), and cardiac output.

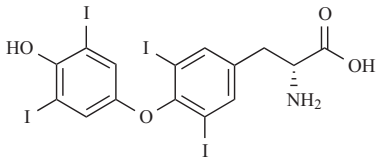

Fig. (4). Thyroxine.

Consequently, case series as well as randomized clinical trials were published on treatment, especially of children, with LOCS using an infusion of T3 in a dosage of 2 or 1 µg/kg/d [66]. Interestingly, those trials, albeit small, showed a significant reduction in composite treatment scores and improved cardiac index, especially in those infants with LCOS after a prolonged time on CPB [67]. In neonates with CHD, who show a high prevalence of thyroid dysfunction in severe illness, the effect of hypothyroidism deserves special attention [68].

The mechanisms of action of T3 in neonates with LOCS are thought to be multiple and considered to be both transcriptional and non transcriptional. The rapid onset effects of T3 in LCOS are most probably again the result of an increased intracellular Ca^{2+} concentration [69].

Transcriptional thyroid hormone-responsive gene expression is thought to include up-regulation of the contractile proteins (α myosin heavy chains) and calcium transport proteins (sarcoplasmic reticulum calcium adenosine triphosphatase), β adrenergic receptors, guanine nucleotide regulatory proteins, Na^+ / K^+ ATPase and voltage gated potassium channels as well as down regulation of β-myosin heavy chains, phospholamban, adenylyl cyclase V +VI, Na^+ / Ca^+ exchanger and T3 nuclear receptor α1 [70,71]. In particular, the effect of T3 on phospholamban seems to be of importance for cardiac function since phospoholamban deficient mice did not experience a positive inotropic effect of T3 treatment [72]. Recently, the BNP gene was identified as a target for T3 action in a study on rat cardiomyocytes, where T3 significantly enhanced BNP gene transcription, an action similar to endothelin [73]. An additional effect of thyroid hormones on cardiac function might be an augmentation of the effect of endogenous or exogenous catecholamines.

In a recent study, 97% of investigated centers performing cardiac surgery for CHD used steroids in children and infants prior to CPB. The main rationale for this treatment, either dexamethasone (Fig. **5**) or methylprednisolone, was to blunt the inflammatory response and the developing SIRS as well as to prevent myocardial injury resulting from both [74,75]. However, there is compelling data that the cardiac action of steroids exceed far beyond their anti-– inflammatory properties by direct or indirect signaling in the cardiomyocyte.

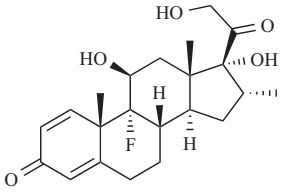

Fig. (5). Dexamethasone.

In neonates, the transition from intra-uterine to extra-uterine life is accompanied by a marked increased in cortisol release from the adrenal glands. The maturating effects of glucocorticoids on organ systems like lung and kidney in the neonatal period are well known and targets for many therapeutic interventions whereas the action of cortisol on the neonatal heart has only recently been elucidated. Most probably, many of the cardiac effects of steroids are mediated through a transcription of genes encoding the ß-subunit of cardiac sodium channels (SCN5a) [76]. These findings implicate that infants delivered prematurely before the physiologic increase in cortisol release, might lack SCN expression which can probably be reversed by cortisol supplementation. There are some reports linking sudden infant death syndrome (SIDS) with mutations in SCN5a, since SIDS has a peak incidence in the 4th month of life when the circadian rhythm of cortisol is established in the human infant [77,78].

Cortison stimulates angiotensinogen (Aogen) expression in the neonatal heart [79]. The renin-angiotensin system (RAS) is important for development and growth of the neonatal heart and neonatal piglets treated with angiotensin converting enzyme (ACE) inhibitors develop a decreased cardiac growth rate [80]. In rat cardiomyocytes, dexamethasone up-regulates expression of Aogen and ACE resulting in a marked hypertrophy of the left ventricle [81]. Still, it is not known if this effect depends solely on the cardiac RAS or if the circulatory / renal RAS is involved.

However, one possible adverse mechanism observed *in vivo*, might be the signaling of glucocorticoids, like mineralocorticoids, through the aldosterone receptor. While the action of aldosterone receptor stimulation with resulting left ventricular hypertrophy is well understood, recent studies revealed induction of cardiomyocyte apoptosis and fibrosis, as well as inflammation by signaling through this receptor which can be stimulated also by glucorticoids [82,83].

Most recently, a report of the successful application of a protocol on the use of hydrocortisone in infants with LCOS after surgery for CHD was published [84]. However, since there are no data of randomized clinical trials on the use of steroids in LOCS in the neonate today, such treatment

should be applied other than in controlled studies, if at all, with extreme caution.

FUTURE PERSPECTIVES

As described, different intracellular mechanisms and pathways can be targeted in pharmacotherapy to improve left ventricular function. The most intense area of research in treating LOCS at present, however, is the field of gene therapy. Several studies in animal models are addressing this question. Three different pathways play a major role in the recent literature: ß-adrenergic receptor (ß-AR) signalling, intracellular calcium signalling, and anti-apoptotic signalling.

ß-adrenergic receptor (ß-AR) signalling: The classical $ß_1$-adrenergic pathway in the cardiomyocyte is mediated via activation of adenylyl cyclases via G_s, resulting in increased cAMP levels, which further activates protein kinase A (PKA). Activated PKA phosphorylates several downstream targets which are essential for cardiomyogenic contraction. This classical pathway is complemented by a non- classical pathway which interacts with the other ß-adrenergic receptor pathways, most importantly the $ß_2$-AR. Signalling by cardiac ß-receptors has been studied in great detail [85].

In neonates and infants, however, only a few studies have been done to investigate an intracellular pathway, which makes it difficult to extrapolate results from studies in adults. It was shown that in different congenital heart diseases, up- or down regulation of beta-adrenoreceptor subtypes occurs. Furthermore, the coupling of the beta-adrenoreceptor subtypes to downstream signaling cascades also varies in different diseases [86-88]. This leads to a reduced reponse of the neonatal heart to catecholamines. Pathways to restore this cathecholamine-impaired ventricular reaction might be given by gene delivery of receptors or downstream targets.

These propositions have already been tested in animal models and showed promising results. For example, enhancement of cardiac function has been demonstrated after adenoviral-mediated *in vivo* intra-coronary $⇓_2$-AR gene delivery [89,90]. Not only ß-AR gene delivery but also the transfection addressing downstream targets was shown to increase left ventricular function in heart failure [91].

Intracellular calcium signalling: Monitoring of intracellular calcium levels is highly important for the physiological myocardium. A crucial role for Ca^{2+} handling is played by the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) which regulates the uptake of Ca^{2+} in the sarcoplasmatic reticulum. Reduced expression or activity of SERCA leads to increased concentrations of cytosolic Ca^{2+} and impaired release from the sarcoplasmic reticulum [92]. Adenoviral over-expression of SERCA2a restored both systolic and diastolic function to normal levels in a rat model of pressure-overload hypertrophy in transition to heart failure [93] and resulted in a positive inotropic effect [94]. Furthermore, inhibition of phospholamban (PLN), an endogenous inhibitor of SERCA2a, improved LV function and prevented remodelling in a rat model of heart failure [95].

Targeting the calcium release pathway via gene therapy seems to be a promising option. Over-expression or blocking of inhibition of calcium release are options to improve the cardiac function in different animal models.

Both pathways (ß-adrenergic receptor signalling and intracellular calcium signalling) have been shown to improve ventricular function. Neveretheless, in contrast to medical therapy which can be stopped immediately, gene transfer leads to a prolonged activation which might turn out to be problematic. Gene transfer concerning the $ß_2$-AR or intracellular calcium signalling leads to higher levels of consumption of oxygen, intracellular hypoxia and might be followed by cell death and consequent aggravation of heart failure. Since a short time benefit of gene therapy, targeting at $ß_2$-AR or Ca^{2+} signalling, is probably annihilated by this phenomenon, gene therapy promoting cell survival seems to be another promising option.

Anti-apoptotic signalling: The other promising strategy to prevent chronic heart failure is given by the possibility to promote myocardial survival. Therefore, the resistance of cardiomyocytes to apoptosis has to be augmented. This approach can be realized by gene transfer with anti-apoptotic factors. It has been shown that gene transfer of anti-apoptotic factors preserves LV function [96]. Not only anti-apoptotic factors, but also growth factor gene transfer following a myocardial infarction resulted in preserved myocardial function and geometry [97]. Also other factors that are not directly linked to apoptotic/survival pathways were found to protect against cardiomyocyte apoptosis [98,99].

In summary, different approaches for gene therapy have been tested in animal models. The $ß_2$-AR signalling and the intracellular calcium signalling pathways show promising results.

In contrast to direct inotropic gene therapy, anti-apoptotic gene therapy may lead to a lower short time benefit; however, the long-term outcome might be convincing. In the treatment of congenital heart diseases in particular, most importantly in the functional uni-ventricular heart, a postive inotropic therapy without a negative long-term effect is of great interest. In this scenario, anti-apoptotic genes may lead to an augmentation of cardiac function. A combination of this therapy together with direct inotropic drug therapy could be promising. However, application of cardio-myogenic gene therapy has just started and there is a long way to go to the widespread clinical use of this fascinating idea.

CONCLUSION

Although enormous improvements in the support of the failing neonatal heart have been achieved with new compounds, the ideal drug has still to be designed. Asking the pediatric cardiologist, such a substance should incorporate many of the characteristics of levosimendan together with a negative chronotropic function.

Unfortunately, randomized clinical trials in neonates are rarely conducted and therefore most of the unique pharmacologic functions of many drugs in this pediatric population unfortunately remain purely speculative.

ABBREVIATIONS

ACE = Angiotensin converting enzyme

ANP = Atrial natriuretic peptide

Aogen	=	Angiotensinogen
ATP	=	Adenosine tri-phosphate
BNP	=	Brain natriuretic peptide
Ca	=	Calcium
cAMP	=	Cyclic adenosine mono-phosphate
cGMP	=	Cyclic guanosine 3,5 mono-phosphate
CHD	=	Congenital heart disease
CNP	=	c Type natriuretic peptide
CO	=	Carbon monoxide
CPB	=	Cardio pulmonary bypass
Km	=	Michaelis Menten konstante
LIDO	=	Low output heart failure study
LOCS	=	Low cardiac output sysndrome
LV	=	Left ventricle
NFκB	=	Nuclear factor kappa B
NO	=	Nitric oxide
NPR	=	Natriuretic peptide receptor
PCWP	=	Pulmonary capillary wedge pressure
PDE	=	Phosphodiesterase
PKA	=	Protein kinase A
PLN	=	Phospholamban
RAS	=	Renin-angiotensin system (RAS)
RUSSLAN	=	Randomized study on safety and effectiveness of levosimendan in patients with left ventricular failure
SCN	=	Cardiac sodium channels
SERCA	=	Sarcoplasmic reticulum Ca^{2+}-ATPase
SIDS	=	Sudden infant death syndrome
SIRS	=	Systemic inflammatory response syndrome
ß$_2$-AR	=	ß$_2$-adrenal receptor
T3	=	Triiodothyronine

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