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Endothelin receptor antagonism in single ventricle physiology with fontan palliation: A systematic review and meta-analysis

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ABSTRACT

Background: The prevalence of single ventricle patients palliated with Fontan operation continues to grow worldwide. This study systematically reviewed existing evidence and performed a meta-analysis to determine the safety and efficacy of endothelin receptor antagonism in single ventricle physiology with Fontan palliation.
Methods: Keyword and reference search was conducted in PubMed Cochrane Library, Web of Science, Google Scholar, and ClinicalTrials.gov databases. Inclusion criteria were — study design: randomized controlled trials, cohort studies, prospective studies, or retrospective studies; subjects: single ventricle patients with Fontan palliation; main outcome: exercise or functional capacity; language: English; and article type: peer-reviewed publications.
Results: Five studies met the inclusion criteria, including three pre–post studies, one randomized crossover open label clinical trial, and one double-blind randomized controlled clinical trial. Study durations ranged from 3.5 to 6 months, with a total sample size of 123. Bosentan was the single endothelin receptor blocker used in all studies. No significant increase in liver toxicity or other serious adverse events were reported in these studies. Meta-analysis found bosentan use to be associated with improvement in functional class ($p = 0.0007$); whereas no significant change in six-minute walk distance, resting oxygen saturation, and maximal oxygen consumption was identified.
Conclusions: Bosentan was found to be a safe and well tolerated endothelin receptor antagonist in Fontan patients over 3–6 months of therapy. Bosentan use was associated with improved functional capacity. Future studies with larger sample size and longer duration are warranted to examine the long-term safety and efficacy of endothelin blockade in Fontan physiology.

1. Introduction

The prevalence of single ventricle patients palliated with the Fontan operation continues to grow worldwide [1–4]. Fontan patients' long-term survival and exercise capacity are largely dependent upon their pulmonary vascular resistance [5–7]. Pulmonary vascular constriction has been linked to the surface endothelin-1 receptors on the pulmonary vascular bed [8]. While endothelin receptor antagonists have been shown to improve hemodynamics and exercise capacity in patients with pulmonary arterial hypertension, [9–11] their efficacy has yet to be assessed in patients with Fontan physiology. This study systematically reviewed existing evidence and performed a meta-analysis to determine the safety and efficacy of endothelin receptor antagonism in single ventricle physiology with Fontan palliation.

2. Methods

2.1. Study selection criteria

Studies that met the following inclusion criteria were included in this review — study design: randomized controlled trials, cohort studies, prospective studies, or retrospective studies; subjects: single ventricle patients with Fontan palliation; main outcome: exercise or functional capacity; language: English; and article type: peer-reviewed publications. Studies that examined the effects of endothelin receptor antagonists (ERAs) in congenital heart disease population other than Fontan patients were excluded from the review. Case reports were also excluded.

2.2. Search strategy

Keyword search was conducted in PubMed, Cochrane Library, Google Scholar and ClinicalTrials.gov databases. The search algorithm

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included all possible combinations of keywords from the following three groups: (1) "bosentan", "endothelin blockade", or "endothelin õantagonist"; (2) "Fontan" or "single ventricle physiology"; and (3) "congenital". Titles and abstracts of the articles identified through keyword search were screened against the study selection criteria. Potentially relevant articles were retrieved for evaluation of the full text.

We also conducted a reference list search (i.e., backward search) and cited reference search (i.e., forward search) from full-text articles meeting the study selection criteria. The backward/forward reference search was performed in Web of Science and Google Scholar. No further articles were identified through this process. The search was completed on March 8, 2015.

2.3. Data extraction

A standardized data extraction form was used to collect the following methodological and outcome variables from each included study: first author, publication year, article title, study design, sample size, attrition rate, gender, age range, study duration, dose of ERA, Fontan type, six-minute walk test (6MWT), resting oxygen saturation, New York Heart Association (NYHA) functional classification, maximal oxygen consumption (VO_2 max), cardiopulmonary exercise test duration, ventricular function, cardiac output, brain natriuretic peptide (BNP) test, drug toxicity, adverse event, quality of life, and study inclusion/exclusion criteria.

2.4. Study quality assessment

Two reviewers independently assessed the quality of the selected studies based on 10 dichotomous criteria that were adapted from the National Institute of Health's tools for assessing risk of bias in randomized controlled trials and cohort studies [12]. The criteria include: (1) Was the study a randomized controlled trial? (2) Were key baseline characteristics between intervention/control groups balanced? (3) Was the overall drop-out rate at study endpoint 20% or less of the number allocated to treatment? (4) Did the study specify the exclusion of patients based on use of phosphodiesterase-5 inhibitors, prostonoids, and other pulmonary vasodilators without a sufficient washout period? (5) Were concomitant medications tracked or specified in baseline characteristics? (6) Were study procedures documented in detail in the article? (7) Was the study question or objective clearly stated? (8) Were eligibility/selection criteria for the study population pre-specified and clearly described? (9) Was the sample size sufficiently large to examine outcomes of interest? (10) Were the outcome measures pre-specified, clearly defined, validated, and consistently assessed across study participants? A total study quality score ranging from zero to 10 was obtained for each study by summing up these criteria. Quality score helped measure the strength of the study evidence, but was not used to determine the inclusion of studies.

2.5. Statistical analysis

Meta-analysis was performed on 6MWT, resting oxygen saturation, VO_2 max, and NYHA functional class outcomes. Across-study heterogeneity was assessed using the I^2 statistic, which indicates the percentage of variability in effect estimate that is due to heterogeneity rather than chance. We reported results from both random-effect and fixed-effect models. Paired t-test was performed using the granular functional class data ($n = 21$) provided by Ovaert et al. [11], Bowater et al. [9] and Derk et al. [10]. Ovaert et al. [11] only reported range along with mean difference for certain outcomes. The range rule (standard deviation is approximated by range divided by six) was used to calculate the standard deviation [13]. Publication bias was assessed by the Egger's test [14] and the Begg's test [15]. All statistical analyses were conducted using Stata 14.0 SE version (StataCorp, College Station, TX).

3. Results

3.1. Study selection

As Fig. 1 shows, among a total of 6425 studies identified from keyword search, 6337 of them were excluded in title and abstract screenings. The remaining 88 articles were reviewed in full texts, and 83 of them were excluded for not meeting the study design or population of interest specified in the inclusion criteria. A backward/forward reference search was conducted on the five selected articles, but no additional article that met the inclusion criteria was found.

3.2. Basic characteristics of the included studies

Study characteristics and main findings are summarized in Tables 1 and 2, respectively. All studies are fairly recent work published between 2009 and 2014. Study designs included three prospective pre–post studies (Bowater et al. [9]; Derk et al. [10]; Ovaert et al. [11]), one randomized crossover open label clinical trial (Schuuring et al. [16]) and one double-blind randomized controlled clinical trial (Herbert et al. [17]). Study durations ranged from 3.5 to 6 months, with a total sample size of 123 patients.

3.3. Intervention effectiveness

All studies found bosentan to be safe and well tolerated in Fontan patients. No study reported a significant increase in liver toxicity or liver enzyme level above three times the upper limit or normal level. No serious adverse event was reported during the study follow-up period. Among the three studies that examined BNP as an outcome, two of them (Derk et al. [10]; Schuuring et al. [16]) found no significant change in BNP levels and one (Herbert et al. [17]) reported a significant decrease in BNP levels (-1.9 ng/L; 95% confidence interval [CI] $= -4.9$ ng/L, -0.5 ng/L; $p = 0.028$) for the bosentan treatment group. Herbert el al. [17] reported a small but significant decrease in hemoglobin levels (-0.3 mmol/L; 95% CI $= -0.5$ mmol/L, -0.1 mmol/L; $p = 0.0001$).

In meta-analysis on 6MWT distance, I^2 statistics equals 65.5% ($p = 0.055$). There is some discrepancy regarding the estimated effect of bosentan treatment on 6MWT distance between random-effect and fixed-effect models. The estimated pooled effect size in the random-effect model is 19.765 m (95% CI $= -26.982$ m, 66.512 m; $p = 0.407$), whereas that in the fixed-effect model is 23.146 m (95% CI =

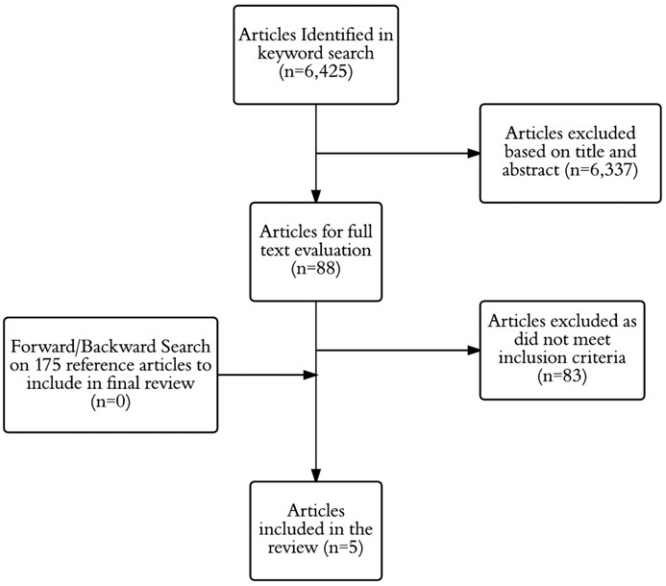

Fig. 1. Study selection flow chart.

Table 1
Characteristics of included studies.

ID	First author	Publication year	Country	Initial sample size	Effective sample size	Attrition rate	Age range (years)	Gender (M/F)
1	Ovaert	2009	Belgium	10	9	10%	4.4–33	7/3
2	Bowater	2012	United Kingdom	8	6	25%	25.2–39.9	3/3
3	Schuuring	2013	Amsterdam	42 Treatment: 21 Placebo: 21	32 Treatment: 32 Placebo: 16	24%	18–55	Treatment: 11/10 Placebo: 11/10
4	Derk	2014	USA	10	7	30%	18–47	3/7
5	Herbert	2014	Denmark	75 Treatment: 36 Placebo: 39	69 Treatment: 32 Placebo: 37	8%	13–28	Treatment: 21/15 Placebo: 24/15

ID	First author	Setting	Study design	Treatment duration (months)	Bosentan dosage	Fontan
1	Ovaert	Multicenter	Prospective single arm	4	62.5 mg BID for 1st month, 125 mg BID for 3 months	3 extracardiac; 7 intracardiac
2	Bowater	Single center	Prospective single arm	6	62.5 mg BID for 2 weeks, then titrated up to 125 mg BID for 3 months	5 RA:PA; 1 TCPC
3	Schuuring	Multicenter	Randomized crossover clinical trial, open label	6	125 mg BID	Treatment: 20 RA:PA; 4 intracardiac; 18 extracardiac Placebo: 11 RA:PA; 2 intracardiac; 8 extracardiac
4	Derk	Single center	Prospective single arm	4	62.5 mg BID for 1st month, 125 mg BID for 3 months	5 RA:PA; 1 intracardiac; 3 extracardiac; 1 LA:PA (fenestrated)
5	Herbert	Multicenter	Double-blind randomized clinical trial	3.5	62.5 mg BID for 2 weeks, then titrated up to 125 mg BID for 3 months	Treatment: 4 RA:PA; 23 intracardiac; 9 extracardiac; Placebo: 2 RA:PA; 22 intracardiac; 15 extracardiac;

ID	First author	Adverse event# (%)	Study inclusion criterion	Study exclusion criterion
1	Ovaert	2 (20) transient fatigue, 1(10) weight gain and edema (discontinued), 2 (20) mild upper airway infection, 1(10) fever due to herpes reactivation	Fontan patients; 2 years or older; heart failure at least 6 months after the Fontan operation; oxygen saturation below 90% at rest or exercise; low output with elevated venous pressure; protein-losing enteropathy; 2,3,4 functional class	Treatable causes of heart failure; pregnant; systolic blood pressure below 80% of normal range; levels of aspartate aminotransferase or alanine aminotransferase 3 times the upper limit of normal; moderate to severe liver disease; hemoglobin or hematocrit below 75% of normal, use of pulmonary vasodilators
2	Bowater	3 (38) reported headache which resolved at 1 month follow-up, 1 (13) leg edema which resolved within first month, 1 (13) thyrotoxicosis secondary to amiodarone therapy (discontinued)	Fontan patients; 18 years or older; NYHA greater than or equal to 2	Lack of sinus rhythm; hematological abnormality; renal dysfunction; pre-existing liver disease; treatment with prostanoids, ERAs, phosphodiesterase type-5 inhibitors within 1 month of study enrollment
3	Schuuring	Treatment group: 3 (7) headache, 1 (2) dyspnea on exertion, 1 (2) peripheral edema, 1 (2) anemia and leukopenia	Fontan patients	Subpulmonary ventricle; moderate or severe liver disease; use of cyclosporine A; pregnancy
4	Derk	1 (10) chest pain (discontinued), 1 (10) palpitations (discontinued), 3 (30) fatigue (discontinued)	Fontan patients; 12 years or older	Pregnancy; use of cyclosporine; levels of aspartate aminotransferase or alanine aminotransferase 3 times the upper limit of normal; contraindications to MRI; use of phosphodiesterase-5 inhibitors or other pulmonary vasodilators
5	Herbert	Treatment group: 1 (3) chest pain (discontinued), 1 (3) recurrence of protein-losing enteropathy (discontinued); 1(3) influenza (discontinued); 6 (17) flushing Placebo group: 1(3) recurrence of protein-losing enteropathy (discontinued), 1 (3) flushing	Fontan patients; 12 years or older; clinically stable (no change 3 months prior);	NYHA class IV; systolic blood pressure below 80% of normal range; oxygen saturation below 85%; neurological sequelae; liver or renal impairment; patients on pulmonary vasodilators

Notes: RA: right atrium, LA: left atrium, PA: pulmonary artery, TCPC: total cavopulmonary connection.

6.725, 39.566; p = 0.006). We would prefer random-effect model estimate because I^2 statistic is greater than 50%, the conventional range for substantial across-study heterogeneity.

Bosentan treatment did not demonstrate any significant effect on resting oxygen saturation. The I^2 statistics in meta-analysis equals 44.3% (p = 0.166). The estimated pooled effect size in the fixed-effect model is −0.448% (95% CI = −2.113%, 1.216%; p = 0.598), and that in the random-effect model is 0.633% (95% CI = −2.52%, 3.786%; p = 0.694).

No significant effect on VO_2 max was found for bosentan treatment. The I^2 statistics in meta-analysis equals 22.5% (p = 0.276). The estimated pooled effect size in the fixed-effect model is −0.643 ml/kg/min (95% CI = −0.272 ml/kg/min, 1.559 ml/kg/min; p = 0.168), and that in the random-effect model is 0.502 ml/kg/min (95% CI = −0.680 ml/kg/min, 1.684 ml/kg/min; p = 0.405).

Two out of the five studies (Bowater et al. [9]; Herbert et al. [17]) found bosentan treatment to be associated with significant improvement in functional class. Paired t-test using the combined granular functional class data (n = 21) from the other three studies (Bowater et al. [9]; Derk et al. [10]; Ovaert et al. [11]) estimates the mean change in functional class to be 0.523 improvement (95% CI = 0.392, 0.654; p = 0.0007) following bosentan treatment.

Neither the Egger's test nor the Begg's test is statistically significant, indicating the absence of publication bias or lack of statistical power to detect such bias.

Table 2
Summary of study findings.

ID	First author	6MWT distance (mean difference ± SE)	Resting oxygen saturation (mean difference ± SE)	NYHA functional class	VO_2 max from stress test (mean difference ± SE)
1	Ovaert	(−35.5 m ± 30.368 m) 95% CI [−95.02, 24.02]; p = 0.27	(+3.0% points ± 2.61% points) 95% CI [−2.2, 8.2]; p = 0.16	4 improved 1 functional class, 5 unchanged; p = NS	(−0.05 ml/min/kg ± 1.512 ml/min/kg) 95% CI [−3.01, 2.91]; p = 0.9747
2	Bowater	(+25 m ± 9 m) 95% CI [7.36, 42.64]; p = 0.07	(−1.2% points ± 0.937% points) 95% [−3.04, 0.64]; p = 0.5827	5 improved 1 functional class; 4 improved from 2 to 1, 1 improved from 3 to 2, 1 remained at 2; p = 0.04	(−0.4 ml/min/kg ± 5.174 ml/min/kg) 95% CI [−9.74, 10.54]; p = 0.9409
3	Schuuring	NA	NA	Treatment group: 6 improved, 24 unchanged, 2 declined; placebo group: 15 unchanged, 1 declined; p = NS	(−0.6 mL/kg/min ± 0.85 mL/kg/min) 95% CI [−2.3, 1.2]; p = 0.63
4	Derk	(+73 m ± 35 m) 95% CI [4.40, 141.60]; p = 0.03	(+3% points ± 3.14% points) 95% CI [−3.15, 9.15]; p = 0.18	3 improved, 3 unchanged, 1 declined; p = NS	NA
5	Herbert	NA	NA	Treatment group: 9 improved 1 FC and none deteriorated; placebo group: 0 improved, 1 declined; ODDS ratio: 0.069; 95% CI [0.0, 0.41]; p = 0.0085	(+1.39 mL/kg/min ± 0.606 mL/kg/min) 95% CI [0.18, 2.59]; p = 0.0245

ID	First author	Cardiopulmonary exercise test duration	Ventricular function	Cardiac output	BNP	Toxicity
1	Ovaert	Reported for one patient: improved from 3 to 8 min	NA	NA	NA	NA
2	Bowater	NA	Significant increase in ventricular systolic annular velocity by 1.8 ± 2.1; p = 0.009	NA	NA	No significant difference in AST, creatinine, potassium, hemoglobin, platelet count levels
3	Schuuring	NA	NA	(+0.2 L/min) 95% CI [−0.3, 0.8] p = 0.46	(−13 ng/L) 95% CI [284, 259]; p = 0.19	No significant difference in creatinine, albumin, sodium, and hemoglobin
4	Derk	NA	Significant increase in MRI-derived cardiac output 7.1 L/min ± 2.8 L/min; p = 0.04	+1.1 L/min ± 0.23 L/min, p = 0.03	(+6.72 ng/L) 95% CI [−32.642, 19.202]; p = 0.61	No significant difference in AST, ALT, total bilirubin
5	Herbert	+0.39 min; 95% CI [0.01, 0.77]; p = 0.042	NA	NA	−1.9 ng/L; 95% CI [−4.9, −0.5]; p = 0.028	No significant hepatotoxicity. Significant decrease in hemoglobin −0.3; 95% CI [−0.5, −0.1]; p = 0.0001

Notes: NA: test not done or not reported, NS: not significant (actual value not reported), SE: standard error. The range rule was used to calculate the standard deviation if not reported: range/6 = standard deviation.

3.4. Study quality assessment

Table 3 reports results from the study quality assessment. Studies included in the review on average met seven out of 10 quality criteria, but the distribution of qualification differed substantially across criteria. Only two studies had balanced key baseline characteristics between intervention/control groups and an overall drop-out rate of 20% or less; whereas all articles documented study procedures in detail, clearly stated study hypothesis or objective, and pre-specified eligibility/selection criteria for the population under investigation.

4. Discussion

Evidence from this systematic review and meta-analysis suggests that bosentan is safe and well tolerated in patients with Fontan circulation. Fontan patients are at increased risk for liver disease due to chronic venous congestion and low cardiac output [19,20]. No study included in this review found significant increase in liver enzyme level or liver toxicity over 3 to 6 months of bosentan therapy. Moreover, bosentan use was found to be significantly improves Fontan patients' functional capacity. However, the long-term safety and tolerability of bosentan and other endothelin blockers, especially regarding liver toxicity, still warrant investigation. Herbert el al. [18] reported a small but significant decrease in hemoglobin levels, which is a known side-effect of bosentan.

Evidence regarding the effect of bosentan use on improvement in six-minute walk distance is inconclusive. The fixed-effect but not the random-effect estimate (which is preferred) found a statistically significant increase in this outcome measure. It is also important to note the substantial variability of 6MWTs as a measure for submaximal exercise capacity. The number of 6MWTs a patient performs tends to be positively associated with his or her six-minute walk distance, with the largest increase in distance observed during the first three trials [20]. It is thus recommended to perform at least two 6MWTs for each data point with results compared. If the between-test variations are less than 10%, the test may be considered a valid measure for a patient's functional capacity [19]. However, none of the studies included in this review assessed or reported the between-test variability of the 6MWT.

Table 3
Study quality assessment.

Criterion	Proportion of studies that meet criterion (%)
Was the study a randomized controlled trial?	2/5 (40%)
Were key baseline characteristics between intervention/control groups balanced?	1/5 (20%)
Was the overall drop-out rate at study endpoint 20% or less of the number allocated to treatment?	2/5 (40%)
Did the study specify the exclusion of patients based on phosphodiesterase-5 inhibitors, prostonoids, and other pulmonary vasodilators?	4/5 (80%)
Were concomitant medications tracked or specified in baseline characteristics?	4/5 (80%)
Were study procedures documented in detail in the article?	5/5 (100%)
Was the study question or objective clearly stated?	5/5 (100%)
Were eligibility/selection criteria for the study population pre-specified and clearly described?	5/5 (100%)
Was the sample size sufficiently large to examine outcomes of interest?	2/5 (40%)
Were the outcome measures pre-specified, clearly defined, validated, and consistently assessed across study participants?	5/5 (100%)

Despite the lack of statistical significance in certain exercise capacity measures like VO_2 max and resting oxygen saturation, bosentan therapy appears to show clinically significant results in some Fontan patients [9–11,17]. The challenge remains to identify characteristics that may predict which patients benefit more (or less) from bosentan therapy.

5. Limitations

A few limitations in the reviewed studies should be noted. Most studies had small sample size, which is common for research on congenital heart disease populations. Attrition rate was higher than 20% for three of the five studies included in the review, which may compromise treatment effect estimation. Studies in the review varied substantially by their primary outcome measures, making meaningful comparisons difficult or infeasible. Granular data was available for only a limited number of studies and outcomes, so that additional assumptions on the data distribution had to be made in order to conduct meta-analysis.

6. Conclusion

Bosentan was found to be a safe and well tolerated endothelin receptor antagonist in Fontan patients over 3 to 6 months of therapy. Bosentan use was associated with improved functional capacity. Future studies with larger sample size and longer duration are warranted to examine the long-term safety and efficacy of endothelin blockade in Fontan physiology.

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